(19) Europäisches Patentamt
European Patent Offic
Office européen des brevets

(11) EP 0 727 487 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
21.08.1996 Bulletin 1996/34

(21) Application number: **95201951.1**

(22) Date of filing: **14.07.1995**

(51) Int. Cl.[6]: **C12N 15/12**, C12N 15/11, C07K 14/47, C12Q 1/68, C07K 19/00, C07K 16/18, A61K 38/17

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **17.02.1995 EP 95200390**

(71) Applicant: **K.U. LEUVEN RESEARCH & DEVELOPMENT**
B-3000 Leuven (BE)

(72) Inventors:
- **Bullerdiek, Jörn**
  D-2800 Bremen (DE)
- **Van de Ven, Willem Jan Marie**
  B-3051 Oud-Heverlee (BE)
- **Schoenmakers, Henricus F.P.M.**
  B-3000 Leuven (BE)
- **Mols, Rafael**
  B-2220 Hallaar (Heist op den Berg) (BE)

(74) Representative: **Van Someren, Petronella F. H. M.**
Arnold & Siedsma,
Advocaten en Octrooigemachtigden,
Sweelinckplein 1
2517 GK Den Haag (NL)

Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Multiple-tumor aberrant growth genes

(57) The present invention relates to the multi-tumor Aberrant Growth (MAG) gene having the nucleotide sequence of any one of the strands of any one of the members of the High Mobility Group protein genes or LIM protein genes, including modified versions thereof. The gene and its derivatives may be used in various diagnostic and therapeutic applications.

EP 0 727 487 A1

Printed by Rank Xerox (UK) Business Services
2.13.2/3.4

involved in non-physiological proliferative growth, and in particular involved in malignant or benign tumors, including atherosclerotic plaques.

The term "wildtyp cell" is used to indicate the cell not harbouring an aberrant chromosome. "Wildtype" or "normal" chromosome refers to a non-aberrant chromosome.

Diagnostic methods are based on the fact that an aberration in a chromosome results in a detectable alteration in the chromosomes' appearance or biochemical behaviour. A translocation, for example will result in a first part of the chromosome (and consequently of the MAG gene) having been substituted for another (second) part (further referred to as "first and second substitution parts"). The first part will often appear someplace else on another chromosome from which the second part originates. As a consequence hybrids will be formed between the remaining parts of both (or in cases of triple translocations, even more) chromosomes and the substitution parts provided by their translocation partners. Since it has now been found that the breaks occur in a MAG gene this will result in hybrid gene products of that MAG gene. Markers, such as hybridising molecules like RNA, DNA or DNA/RNA hybrids, or antibodies will be able to detect such hybrids, both on the DNA level, and on the RNA or protein level.

For example, the transcript of a hybrid will still comprise the region provided by the remaining part of the gene/chromosome but will miss the region provided by the substitution part that has been translocated. In the case of inversions, deletions and insertions the gene may be equally afflicted.

Translocations are usually also cytogenetically detectable. The other aberrations are more difficult to find because they are often not visible on a cytogenetical level. The invention now provides possibilities for diagnosing all these types of chromosomal aberrations.

In translocations markers or probes based on the MAG gene for the remaining and substitution parts of a chromosome in situ detect the remaining part on the original chromosome but the substitution part on another, the translocation partner.

In the case of inversions for example, two probes will hybridise at a specific distance in the wildtype gene. This distance might however change due to an inversion. In situ such inversion may thus be visualized by labeling a set of suitable probes with the same or different detectable markers, such as fluorescent labels. Deletions and insertions may be detected in a similar manner.

According to the invention the above in situ applications can very advantageously be performed by using FISH techniques. The markers are e.g. two cosmids one of which comprises exons 1 to 3 of the MAG gene, while the other comprises exons 4 and 5. Both cosmids are labeled with different fluorescent markers, e.g. blue and yellow. The normal chromosome will show a combination of both labels, thus giving a green signal, while the translocation is visible as a blue signal on the remaining part of one chromosome (e.g. 12) while the yellow signal is found on another chromosome comprising the substitution part. In case the same labels are used for both probes, the intensity of the signal on the normal chromosome will be 100%, while the signal on the aberrant chromosomes is 50%. In the case of inversions one of the signals shifts from one place on the normal chromosome to another on the aberrant one.

In the above applications a reference must be included for comparison. Usually only one of the two chromosomes is afflicted. It will thus be very convenient to use the normal chromosome as an internal reference. Furthermore it is important to select one of the markers on the remaining or unchanging part of the chromosome and the other on the substitution or inverted part. In the case of the MAG gene of chromosome 12, breaks are usually found in the intron between exons 3 and 4 as is shown by the present invention. Probes based on exons 1 to 3 and 4 and 5 are thus very useful. As an alternative a combination of probes based on both translocation or fusion partners may be used. For example, for the identification of lipomas one may use probes based on exons 1 to 3 of the HMGI-C gene on the one hand and based on the LIM domains of the LPP gene on the other hand.

"Probes" as used herein should be widely interpreted and include but are not limited to linear DNA or RNA strands, Yeast Artificial Chromosomes (YACs), or circular DNA forms, such as plasmids, phages, cosmids etc..

These in situ methods may be used on metaphase and interphase chromosomes.

Besides the above described in situ methods various diagnostic techniques may be performed on a more biochemical level, for example based on alterations in the DNA, RNA or protein. Basis for these methods is the fact that by choosing suitable probes, variations in the length or composition in the gene, transcript or protein may be detected on a gel or blot. Variations in length are visible because the normal gene, transcript(s) or protein(s) will appear in another place on the gel or blot then the aberrant one(s). In case of a translocation more than the normal number of spots will appear.

Based on the above principles the invention thus relates to a method of diagnosing cells having a non-physiological proliferative capacity, comprising the steps of taking a biopsy of the cells to be diagnosed, isolating a suitable MAG gene-related macromolecule therefrom, and analysing the macromolecule thus obtained by comparison with a reference molecule originating from cells not showing a non-physiological proliferative capacity, preferably from the same individual. The MAG gene-related macromolecule may thus be a DNA, an RNA or a protein. The MAG gene may be either a member of the HMG family or of the LIM family.

In a specific embodiment the diagnostic method of the invention comprises the steps of taking a biopsy of the cells to be diagnosed, extracting total RNA thereof, preparing a first strand cDNA of the mRNA species in the total RNA

extract or poly-A-selected fraction(s) thereof, which cDNA comprises a suitable tail; performing a PCR using a MAG gene specific primer and a tail-specific primer in order to amplify MAG gene specific cDNA's; separating the PCR products on a gel to obtain a pattern of bands; evaluating the presence of aberrant bands by comparison to wildtype bands, preferably originating from the same individual.

As an alternative amplification may be performed by means of the Nucleic Acid Sequence-Based Amplification (NASBA) technique [81] or variations thereof.

In another embodiment the method comprises the steps of taking a biopsy of the cells to be diagnosed, isolating total protein therefrom, separating the total protein on a gel to obtain essentially individual bands, optionally transfering the bands to a Western blot, hybridising the bands thus obtained with antibodies directed against a part of the protein encoded by the remaining part of the MAG gene and against a part of the protein encoded by the substitution part of the MAG gene; visualising the antigen-antibody reactions and establishing the presence of aberrant bands by comparison with bands from wildtype proteins, preferably originating from the same individual.

In a further embodiment the method comprises taking a biopsy of the cells to be diagnosed; isolating total DNA therefrom; digesting the DNA with one or more so-called "rare cutter" (typically "6- or more cutters") restriction enzymes; separating the digest thus prepared on a gel to obtain a separation pattern; optionally transfering the separation pattern to a Southern blot; hybridising the separation pattern in the gel or on the blot with a set of probes under hybridising conditions; visualising the hybridisations and establishing the presence of aberrant bands by comparison to wildtype bands, preferably originating from the same individual.

The diagnostic method of the invention may be used for diseases wherein cells having a non-physiological proliferative capacity are selected from the group consisting of benign tumors, such as the mesenchymal tumors hamartomas (e.g. breast and lung), lipomas, pleomorphic salivary gland adenomas, uterine leiomyomas, angiomyxomas, fibroadenomas of the breast, polyps of the endometrium, atherosclerotic plaques, and other benign tumors as well as various malignant tumors, including but not limited to sarcomas (e.g. rhabdomyosarcoma, osteosarcoma) and carcinomas (e.g. of breast, lung, skin, thyroid).

Recent publications indicate that atherosclerotic plaques also involve abnormal proliferation [26] and it was postulated that atherosclerotic plaques constitute benign tumors [27]. Therefore, this type of disorder is also to be understood as a possible indication for the use of the MAG gene family, in particular in diagnostic and therapeutic applications.

It has been found that in certain malignant tumors the expression level of the HMG genes is increased [28]. Another aspect of the invention thus relates to the implementation of the identification of the MAG genes in therapy. The invention for example provides anti-sense molecules or expression inhibitors of the MAG gene for use in the treatment of diseases involving cells having a non-physiological proliferative capacity by modulating the expression of the gene.

The invention thus provides derivatives of the MAG gene for use in diagnosis and the preparation of therapeutical compositions, wherein the derivatives are selected from the group consisting of sense and anti-sense cDNA or fragments thereof, transcripts of the gene or fragments thereof, triple helix inducing molecule or other types of "transcription clamps", fragments of the gene or its complementary strand, proteins encoded by the gene or fragments thereof, protein nucleic acids (PNA), antibodies directed to the gene, the cDNA, the transcript, the protein or the fragments thereof, as well as antibody fragments.

It is to be understood that the principles of the present invention are described herein for illustration purposes only with reference to the HMGI-C gene mapping at chromosome 12 and the LPP gene on chromosome 3. Based on the information provided in this application the skilled person will be able to isolate and sequence corresponding genes of the gene family and apply the principles of this invention by using the gene and its sequence without departing from the scope of the general concept of this invention.

The present invention will thus be further elucidated by the following examples which are in no way intended to limit the scope thereof.

EXAMPLES

EXAMPLE 1

1. Introduction

This example describes the isolation and analysis of 75 overlapping YAC clones and the establishment of a YAC contig (set of overlapping clones), which spans about 6 Mb of genomic DNA around locus D12S8 and includes MAR. The orientation of the YAC contig on the long arm of chromosome 12 was determined by double-color FISH analysis. On the basis of STS-content mapping and restriction enzyme analysis, a long range physical map of this 6 Mb DNA region was established. The contig represents a useful resource for cDNA capture aimed at identifying genes located in 12q15, including the one directly affected by th various chromosome 12 aberrations.

2. Materials and methods

2.1. Cell lines

Cell lines PK89-12 and LIS-3/SV40/A9-B4 were used for Chromosome Assignment using Somatic cell Hybrids (CASH) experiments. PK89-12, which contains chromosome 12 as the sole human chromosome in a hamster genetic background, has been described before [29]. PK89-12 cells were grown in DME-F12 medium supplemented with 10% fetal bovine serum, 200 IU/ml penicillin, and 200 µg/ml streptomycin. Somatic cell hybrid LIS-3/SV40/A9-B4 was obtained upon fusion of myxoid liposarcoma cell line LIS-3/SV40, which carries a t(12;16)(q13;p11.2), and mouse A9 cells and was previously shown to contain der(16), but neither der(12) nor the normal chromosome 12 [30]. LIS-3/SV40/A9-B4 cells were grown in selective AOA-medium (AOA-medium which consisted of DME-F12 medium supplemented with 10% fetal bovine serum, 0.05 mM adenine, 0.05 mM ouabain, and 0.01 mM azaserine). Both cell lines were frequently assayed by standard cytogenetic techniques.

2.2. Nucleotide sequence analysis and oligonucleotides.

Nucleotide sequences were determined according to the dideoxy chain termination method using a T7 polymerase sequencing kit (Pharmacia/LKB) or a dsDNA Cycle Sequencing System (GIBCO/BRL). DNA fragments were subcloned in pGEM-3Zf(+) and sequenced using FITC-labelled standard SP6 or T7 primers, or specific primers synthesized based upon newly obtained sequences. Sequencing results were obtained using an Automated Laser Fluorescent (A.L.F.) DNA sequencer (Pharmacia Biotech) and standard 30 cm, 6% Hydrolink^{R}, Long Range™ gels (AT Biochem). The nucleotide sequences were analyzed using the sequence analysis software Genepro (Riverside Scientific), PC/Gene (IntelliGenetics), the IntelliGenetics Suite software package (IntelliGenetics, Inc.), and Oligo [31]. All oligonucleotides were purchased from Pharmacia Biotech.

2.3. Chromosome preparations and fluorescence in situ hybridization (FISH)

FISH analysis of YAC clones was performed to establish their chromosomal positions and to identify chimeric clones. FISH analysis of cosmid clones corresponding to STSs of YAC insert ends were performed to establish their chromosomal positions. Cosmids were isolated from human genomic library CMLW-25383 [32] or the arrayed chromosome 12-specific library constructed at Lawrence Livermore National Laboratory (LL12NC01, ref. 33) according to standard procedures [34]. Routine FISH analysis was performed essentially as described before [30, 35]. DNA was labelled with biotin-11-dUTP (Boehringer) using the protocol of Kievits et al. [36]. Antifade medium, consisting of DABCO (2 g/100 ml, Sigma), 0.1 M Tris-HCL pH 8, 0.02% Thimerosal, and glycerol (90%), and containing propidium iodide (0.5 µg/ml, Sigma) as a counterstain, was added 15 min before specimens were analyzed on a Zeiss Axiophot fluorescence microscope using a double band-pass filter for FITC/Texas red (Omega Optical, Inc.). Results were recorded on Scotch (3M) 640 ASA film.

For the double colour FISH experiments, LLNL12NCO1-96C11 was labelled with digoxygenin-11-dUTP (Boehringer) and cosmids LLNL12NCO1-1F6 and -193F10, with biotin-11-dUTP. Equal amounts of each probe were combined and this mixture was used for hybridization. After hybridization, slides were incubated for 20 min with Avidin-FITC and then washed as described by Kievits et al. [36]. Subsequent series of incubations in TNB buffer (0.1 M Tris-HCl pH 7.5, 0.15 M NaCl, 0.5% Boehringer blocking agent (Boehringer)) and washing steps were performed in TNT buffer (0.1 M Tris-HCl pH 7.5, 0.15 M NaCl, 0.05% Tween-20); all incubations were performed at 37 °C for 30 min. During the second incubation, Goat-α-Avidin-biotin (Vector) and Mouse-α-digoxygenin (Sigma) were applied simultaneously. During the third incubation, Avidin-FITC and Rabbit-α-Mouse-TRITC (Sigma) were applied. During the last incubation, Goat-α-Rabbit-TRITC (Sigma) was applied. After a last wash in TNT buffer, samples were washed twice in 1 x PBS and then dehydrated through an ethanol series (70%, 90%, 100%). Antifade medium containing 75 ng/µl DAPI (Serva) as counterstain was used. Specimens were analyzed on a Zeiss Axiophot fluorescence microscope as described above.

2.4. Screening of YAC libraries.

YAC clones were isolated from CEPH human genomic YAC libraries mark 1 and 3 [37, 38] made available to us by the Centre d'Étude du Polyphormisme Humain (CEPH). Screening was carried out as previously described [39]. Contaminating Candida parapsylosis, which was sometimes encountered, was eradicated by adding terbinafin (kindly supplied by Dr. Dieter Römer, Sandoz Pharma LTD, Basle, Switzerland) to the growth medium (final concentration: 25 µg/ml). The isolated YAC clones were characterized by STS-content mapping, contour-clamped homogeneous electric field (CHEF) gel lectrophoresis [40], restriction mapping, and hybridization- and FISH analysis.

2.5. PCR reactions

PCR amplification was carried out using a Pharmacia/LKB Gene ATAQ Controller (Pharmacia/LKB) in final volumes of 100 µl containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatine, 2 mM dNTP's, 20 pmole of each amplimer, 2.5 units of Amplitaq (Perkin-Elmer Cetus), and 100 ng (for superpools) or 20 ng (for pools) of DNA. After initial denaturation for 5 min at 94 °C, 35 amplification cycles were performed each consisting of denaturation for 1 min at 94 °C, annealing for 1 min at the appropriate temperature (see Table I) and extension for 1 min at 72 °C. The PCR reaction was completed by a final extension at 72 °C for 5 min. Results were evaluated by analysis of 10 µl of the reaction product on polyacrylamide minigels.

2.6. Pulsed-field gel electrophoresis and Southern blot analysis

Pulsed-field gel electrophoresis and Southern blot analysis were performed exactly as described by Schoenmakers et al. [39]. Agarose plugs containing high-molecular weight YAC DNA (equivalent to about 1 x 10^8 yeast cells) were twice equilibrated in approximately 25 ml TE buffer (pH 8.0) for 30 min at 50 °C followed by two similar rounds of equilibration at room temperature. Plugs were subsequently transferred to round-bottom 2 ml eppendorf tubes and equilibrated two times for 30 min in 500 µl of the appropriate 1 x restriction-buffer at the appropriate restriction temperature. Thereafter, DNA was digested in the plugs according to the suppliers (Boehringer) instructions for 4 h using 30 units of restriction endonuclease per digestion reaction. After digestion, plugs along with appropriate molecular weight markers were loaded onto a 1% agarose / 0.25 x TBE gel, sealed with LMP-agarose and size fractionated on a CHEF apparatus (Biorad) for 18 h at 6.0 V/cm using a pulse angle of 120 degrees and constant pulse times varying from 10 sec (separation up to 300 kbp) to 20 sec (separation up to 500 kbp). In the case of large restriction fragments, additional runs were performed, aiming at the separation of fragments with sizes above 500 kbp. Electrophoresis was performed at 14 °C in 0.25 x TBE. As molecular weight markers, lambda ladders (Promega) and home-made plugs containing lambda DNA cut with restriction endonuclease HindIII were used. After electrophoresis, gels were stained with ethidium bromide, photographed, and UV irradiated using a stratalinker (Stratagene) set at 120 mJ. DNA was subsequently blotted onto Hybond N^+ membranes (Amersham) for 4-16 h using 0.4 N NaOH as transfer buffer. After blotting, the membranes were dried for 15 min at 80 °C, briefly neutralised in 2 x SSPE, and prehybridised for at least 3 h at 42 °C in 50 ml of a solution consisting of 50% formamide, 5 x SSPE, 5 x Denhardts, 0.1% SDS and 200 µg/ml heparin. Filters were subsequently hybridised for 16 h at 42 °C in 10 ml of a solution consisting of 50% formamide, 5 x SSPE, 1 x Denhardts, 0.1% SDS, 100 µg/ml heparin, 0.5% dextran sulphate and 2-3 x 10^6 cpm/ml of labelled probe. Thereafter, membranes were first washed two times for 5 min in 2 x SSPE/0.1% SDS at room temperature, then for 30 min in 2 x SSPE/0.1% SDS at 42 °C and, finally, in 0.1 x SSPE/0.1% SDS for 20 min at 65 °C. Kodak XAR-5 films were exposed at - 80 °C for 3-16 h, depending on probe performance. Intensifying screens (Kyokko special 500) were used.

2.7. Generation of STSs from YAC insert ends

STSs from YAC insert ends were obtained using a vectorette-PCR procedure in combination with direct DNA sequencing analysis, essentially as described by Geurts et al. [41]. Primer sets were developed and tested on human genomic DNA, basically according to procedures described above. STSs will be referred to throughout this application by their abbreviated names (for instance: RM1 instead of STS 12-RM1) for reasons of convenience.

3. Results

3.1. Assembly of a YAC Contig around locus D12S8

In previous studies [39], a 800 kb YAC contig around D12S8 was described. This contig consisted of the following three partially overlapping, non-chimeric CEPH YAC clones: 258F11, 320F6, and 234G11. This contig was used as starting point for a chromosome walking project to define the DNA region on the long arm of chromosome 12 that encompasses the breakpoints of a variety of benign solid tumors, which are all located proximal to D12S8 and distal to CHOP. Initially, chromosome walking was performed bidirectionally until the size of the contig allowed reliable determination of the orientation of it. In the bidirectional and subsequent unidirectional chromosome walking steps, the following general procedure was used. First, rescuing and sequencing the ends of YAC clones resulted in DNA markers characterizing the left and right sides of these (Table I). Based on sequence data of the ends of forty YAC inserts, primer sets were developed for specific amplification of DNA; establishing STSs (Table II). Their localization to 12q13-qter was determined by CASH as well as FISH after corresponding cosmid clones were isolated. It should be noted that isolated YAC clones were often evaluated by FISH analysis too, thus not only revealing the chromosomal origin of their inserts but also, for a number of cases, establishing and defining their chimeric nature. Moreover, it should be emphasized that data obtained by restriction endonuclease analysis of overlapping YAC clones were also taken into account in the YAC

clone evaluation and subsequent alignment. With the sequentially selected and evaluated primer sets, screening of the YAC and cosmid libraries was performed to isolate the building blocks for contig-assembly. Therefore, contig-ass mbly was performed using data derived from FISH- and STS-content mapping as well as restriction endonuclease analysis. Using this approach, we established a YAC contig consisting of 75 overlapping YAC clones, covering approximately 6 Mb of DNA (Fig. 1). This contig appeared to encompass the chromosome 12 breakpoints of all tumor-derived cell lines studied [39]. Characteristics of the YACs that were used to build this contig are given in Table I.

3.2. Establishment of the Chromosomal Orientation of the YAC Contig

To allow unidirectional chromosome walking towards the centromere of chromosome 12, the orientation of the DNA region flanked by STSs RM14 and RM26 (approximate size: 1450 kb) was determined by double-color interphase FISH analysis. Cosmid clones corresponding to these STSs (i.e. LL12NC01-1F6 (RM14) and LL12NC01-96C11 (RM26)) were differentially labelled to show green or red signals, respectively. As a reference locus, cosmid LL12NC01-193F10 was labelled to show green signals upon detection. LL12NC01-193F10 had previously been mapped distal to the breakpoint of LIS-3/SV40 (i.e. CHOP) and proximal to the chromosome 12q breakpoints in lipoma cell line Li-14/SV40 and uterine leiomyoma cell line LM-30.1/SV40. LL12NC01-1F6 and LL12NC01-96C11 were found to be mapping distal to the 12q breakpoints in lipoma cell line Li-14/SV40 and uterine leiomyoma cell line LM-30.1/SV40. Therefore, LL12NC01-193F10 was concluded to be mapping proximal to both RM14 and RM26 (unpublished results). Of 150 informative interphases scored, 18% showed a signal-order of red-green-green whereas 72% showed a signal order of green-red-green. Based upon these observations, we concluded that RM26 mapped proximal to RM14, and therefore we continued to extend the YAC contig from the RM26 (i.e. proximal) side of our contig only. Only the cosmids containing RM14 and RM26 were ordered by double-color interphase mapping; the order of all others was deduced from data of the YAC contig. Finally, it should be noted that the chromosomal orientation of the contig as proposed on the basis of results of the double-color interphase FISH studies was independently confirmed after the YAC contig had been extended across the chromosome 12 breakpoints as present in a variety of tumor cell lines. This confirmatory information was obtained in extensive FISH studies in which the positions of YAC and cosmid clones were determined relative to the chromosome 12q13-q15 breakpoints of primary lipomas, uterine leiomyomas, pleomorphic salivary gland adenomas, and pulmonary chondroid hamartomas or derivative cell lines [24, 42, 25, 43].

3.3. Construction of a Rare-Cutter Physical Map from the 6 Mb YAC Contig around D12S8

Southern blots of total yeast plus YAC DNA, digested to completion with rare-cutter enzymes (see Materials and Methods) and separated on CHEF gels, were hybridized sequentially with i) the STS used for the initial screening of the YAC in question, ii) pYAC4 right arm sequences, iii) pYAC4 left arm sequences, and iv) a human ALU-repeat probe (BLUR-8). The long-range restriction map that was obtained in this way, was completed by probing with PCR-isolated STSs/YAC end probes. Occasionally double-digests were performed.

Restriction maps of individual YAC clones were aligned and a consensus restriction map was established. It is important to note here that the entire consensus rare-cutter map was supported by at least two independent clones showing a full internal consistency.

3.4. Physical mapping of CA repeats and monomorphic STSs/ESTs

Based upon integrated mapping data as emerged from the Second International Workshop on Human Chromosome 12 [44], a number of published markers was expected to be mapping within the YAC contig presented here. To allow full integration of our mapping data with those obtained by others, a number of markers were STS content-mapped on our contig, and the ones found positive were subsequently sublocalized by (primer-)hybridization on YAC Southern blots. Among the markers that were found to reside within the contig presented here were CA repeats D12S313 (AFM207xf2) and D12S335 (AFM273vg9) [45], D12S375 (CHLC GATA3F02), and D12S56 [46]. Furthermore, the interferon gamma gene (IFNG) [47], the ras-related protein gene Rap1B [48], and expressed sequence tag EST01096 [49] were mapped using primer sets which we developed based on publicly available sequence data (see Table II). Markers which were tested and found negative included D12S80 (AFM102xd6), D12S92 (AFM203va7), D12S329 (AFM249xh9) and D12S344 (AFM296xd9).

4. Discussion

In the present example the establishment of a YAC contig and rare-cutter physical map covering approximately 6 Mb on 12q15, a region on the long arm of human chromosome 12 containing MAR in which a number of recurrent chromosomal aberrations of benign solid tumors are known to be mapping was illustrated.

The extent of overlap between individual YACs has been carefully determined, placing the total length of the contig at approximately 6 Mb (Fig. 1). It should be noted that our sizing-data for some of the YAC clones differ slightly from th sizes determined by CEPH [50]. It is our belief that this is most probably due to differences in the parameters for running the pulsed-field gels in the different laboratories.

Using restriction mapping and STS-content analysis, a consensus long range physical map (Fig. 1) was constructed. The entire composite map is supported by at least two-fold coverage. In total over 30 Mb of YAC DNA was characterized by restriction and STS content analysis, corresponding to an average contig coverage of about 5 times. Although the "inborn" limited resolution associated with the technique of pulsed-field electrophoresis does not allow very precise size estimations, comparison to restriction mapping data obtained from a 500 kb cosmid contig contained within the YAC contig presented here showed a remarkable good correlation. Extrapolating from the cosmid data, we estimate the accuracy of the rare-cutter physical map presented here at about 10 kb.

The results of our physical mapping studies allowed integration of three gene-specific as well as five anonymous markers isolated by others (indicated in between arrows in Fig. 1). The anonymous markers include one monomorphic and four polymorphic markers. Five previously published YAC-end-derived single copy STSs (RM1, RM4, RM5, RM7, and RM21) as well as four published CA repeats (D12S56, D12S313, D12S335, and D12S375) and three published gene-associated STSs/ESTs (RAP1B, EST01096, and IFNG) have been placed on the same physical map and this will facilitate (linkage-) mapping and identification of a number of traits/disease genes that map in the region. Furthermore, we were able to place onto the same physical map, seventy two YAC-end-derived (Table I) and eight cosmid-end- or inter-ALU-derived DNA markers (CH9, RM1, RM110, RM111, RM130, RM131, RM132, and RM133), which were developed during the process of chromosome walking. The PYTHIA automatic mail server at PYTHIA@anl.gov was used to screen the derived sequences of these DNA markers for the presence of repeats. For forty three of these seventy two DNA markers (listed in Table II), primer sets were developed and the corresponding STSs were determined to be single copy by PCR as well as Southern blot analysis of human genomic DNA. The twenty nine remaining DNA markers (depicted in the yellow boxes) represent YAC-end-derived sequences for which we did not develop primer sets. These YAC-end sequences are assumed to be mapping to chromosome 12 on the basis of restriction mapping. The final picture reveals an overall marker density in this region of approximately one within every 70 kb.

The analysis of the contig presented here revealed many CpG-rich regions, potentially HTF islands, which are known to be frequently associated with housekeeping genes. These CpG islands are most probably located at the 5' ends of as yet unidentified genes: it has been shown that in 90% of cases in which three or more rare-cutter restriction sites coincide in YAC DNA there is an associated gene [51]. This is likely to be an underestimate of the number of genes yet to be identified in this region because 60% of tissue-specific genes are not associated with CpG islands [52] and also because it is possible for two genes to be transcribed in different orientations from a single island [53].

While several of the YAC clones that were isolated from the CEPH YAC library mark 1 were found to be chimeric, overlapping YAC clones that appeared to be non-chimeric based on FISH, restriction mapping and STS content analysis could be obtained in each screening, which is in agreement with the reported complexity of the library. The degree of chimerism for the CEPH YAC library mark 1 was determined at 18% (12 out of 68) for the region under investigation here. The small number of YACs from the CEPH YAC library mark 3 (only 7 MEGA YACs were included in this study) did not allow a reliable estimation of the percentage of chimeric clones present in this library. The average size of YACs derived from the mark 1 library was calculated to be 381 kb ; non-chimeric YACs (n=58) had an average size of 366 kb while chimeric YACs (n=12) were found to have a considerable larger average size; i.e. 454 kb.

In summary, we present a 6 Mb YAC contig corresponding to a human chromosomal region which is frequently rearranged in a variety of benign solid tumors. The contig links over 84 loci, including 3 gene-associated STSs. Moreover, by restriction mapping we have identified at least 12 CpG islands which might be indicative for genes residing there. Finally, four CA repeats have been sublocalized within the contig. The integration of the genetic, physical, and transcriptional maps of the region provides a basic framework for further studies of this region of chromosome 12. Initial studies are likely to focus on MAR and ULCR12, as these regions contain the breakpoint cluster regions of at least three distinct types of solid tumors. The various YAC clones we describe here are valuable resources for such studies. They should facilitate the search for genes residing in this area and the identification of those directly affected by the chromosome 12q aberrations of the various benign solid tumors.

EXAMPLE 2

1. Introduction

It was found that the 1.7 Mb Multiple Aberration Region on human chromosome 12q15 harbors recurrent chromosome 12 breakpoints frequently found in different benign solid tumor types. In this example the identification of an HMG gene within MAR that appears to be of pathogenetical relevance is described. Using a positional cloning approach, the High Mobility Group protein gene HMGI-C was identified within a 175 kb segment of MAR and its genomic organization characterized. By FISH, within this gene the majority of the breakpoints of seven different benign solid tumor types were

pinpointed. By Southern blot and 3'-RACE analysis, consistent rearrangements in HMGI-C and/or expression of altered HMGI-C transcripts were demonstrated. These results indicate a link between a member of the HMG gene family and benign solid tumor development.

2. Materials and methods

2.1. Cell culture and primary tumor specimens.

Tumor cell lines listed in Fig. 3 were established by a transfection procedure [54] and have been described before in [39, 24] and in an article of Van de Ven et al., Genes Chromosom. Cancer 12, 296-303 (1995) enclosed with this application as ANNEX 1. Cells were grown in TC199 medium supplemented with 20% fetal bovine serum and were assayed by standard cytogenetic techniques at regular intervals. The human hepatocellular carcinoma cell lines Hep 3B and Hep G2 were obtained from the ATCC (accession numbers ATCC HB 8064 and ATCC HB 8065) and cultured in DMEM/F12 supplemented with 4% Ultroser (Gibco/BRL). Primary solid tumors were obtained from various University Clinics.

2.2. YAC and cosmid clones

YAC clones were isolated from the CEPH mark 1 [57] and mark 3 [58] YAC libraries using a combination of PCR-based screening [59] and colony hybridization analysis. Cosmid clones were isolated from an arrayed human chromosome 12-specific cosmid library (LL12NC01) [60] obtained from Lawrence Livermore National Laboratory (courtesy P. de Jong). LL12NC01-derived cosmid clones are indicated by their microtiter plate addresses; i.e. for instance 27E12.

Cosmid DNA was extracted using standard techniques involving purification over Qiagen tips (Diagen). Agarose plugs containing high-molecular weight yeast + YAC DNA (equivalent to 1 x 10^9 cells ml^{-1}) were prepared as described before [61]. Plugs were thoroughly dialysed against four changes of 25 ml $T_{10}E_1$ (pH 8.0) followed by two changes of 0.5 ml 1 x restriction buffer before they were subjected to either pulsed-field restriction enzyme mapping or YAC-end rescue. YAC-end rescue was performed using a vectorette-PCR procedure in combination with direct solid phase DNA sequencing, as described before in reference 61. Inter-Alu PCR products were isolated using published oligonucleotides TC65 or 517 [62] to which SalI-tails were added to facilitate cloning. After sequence analysis, primer pairs were developed using the OLIGO computer algorithm [61].

2.3. DNA labelling

DNA from YACs, cosmids, PCR products and oligonucleotides was labelled using a variety of techniques. For FISH, cosmid clones or inter-Alu PCR products of YACs were biotinylated with biotin-11-dUTP (Boehringer) by nick translation. For filter hybridizations, probes were radio-labelled with α-^{32}P-dCTP using random hexamers [62]. In case of PCR-products smaller than 200 bp in size, a similar protocol was applied, but specific oligonucleotides were used to prime labelling reactions. Oligonucleotides were labelled using γ-^{32}P-ATP.

2.4. Nucleotide sequence analysis and PCR amplification

Nucleotide sequences were determined as described in Example 1. Sequencing results were analyzed using an A.L.F. DNA sequencer™ (Pharmacia Biotech) on standard 30 cm, 6% Hydrolink^R, Long Range™ gels (AT Biochem). PCR amplifications were carried out essentially as described before [39].

2.5. Rapid amplification of cDNA ends (RACE)

Rapid amplification of 3' cDNA-ends (3'-RACE) was performed using a slight modification of part of the GIBCO/BRL 3'-ET protocol. For first strand cDNA synthesis, adapter primer (AP2) AAG GAT CCG TCG ACA TC(T)$_{17}$ was used. For both initial and secondary rounds of PCR, the universal amplification primer (UAP2) CUA CUA CUA CUA AAG GAT CCG TCG ACA TC was used as "reversed primer". In the first PCR round the following specific "forward primers" were used: i) 5'-CTT CAG CCC AGG GAC AAC-3' (exon 1), ii) 5'-CAA GAG GCA GAC CTA GGA-3' (exon 3), or iii) 5'-AAC AAT GCA ACT TTT AAT TAC TG-3' (3'-UTR). In the second PCR round the following specific forward primers (nested primers as compared to those used in the first round) were used: i) 5'-CAU CAU CAU CAU CGC CTC AGA AGA GAG GAC-3' (exon 1), ii) 5'-CAU CAU CAU CAU GTT CAG AAG AAG CCT GCT-3' (exon 4), or iii) 5'-CAU CAU CAU TTG ATC TGA TAA GCA AGA GTG GG-3' (3'-UTR). CUA/CAU-tailing of the nested, specific primers allowed the use of the directional CloneAmp cloning system (GIBCO/BRL).

3. Results

3.1. Development of cosmid contig and STS map of MAR segment

During the course of a positional cloning effort focusing on the long arm of human chromosome 12, we constructed a yeast artificial chromosome (YAC) contig spanning about 6 Mb and consisting of 75 overlapping YACs. For a description thereof reference is made to Example 1. This contig encompasses MAR (see also Fig. 2), in which most of the chromosome 12q13-q15 breakpoints as present in a variety of primary benign solid tumors (34 tumors of eight different types tested sofar; Table 5) and tumor cell lines (26 tested sofar, derived from lipoma, uterine leiomyoma, and pleomorphic salivary gland adenoma; Fig. 3) appear to cluster. We have developed both a long-range STS and rare cutter physical map of MAR and found, by FISH analysis, most of the breakpoints mapping within the 445 kb subregion of MAR located between STSs RM33 and RM98 (see Fig. 2 and 3). FISH experiments, including extensive quality control, were performed according to routine procedures as described before [25, 39, 24, 42, 36] To further refine the distribution of breakpoints within this 445 kb MAR segment, a cosmid contig consisting of 54 overlapping cosmid clones has been developed and a dense STS map (Fig. 2) established. The cosmid contig was double-checked by comparison to the rare cutter physical map and by STS content mapping.

3.2. Clustering of the chromosome 12q breakpoints within a 175 kb DNA segment of MAR

The chromosome 12q breakpoints in the various tumor cell lines studied was pinpointed within the cosmid contig by FISH (Fig. 3). As part of our quality control FISH experiments [25, 39, 24, 42], selected cosmids were first tested on metaphase spreads derived from normal lymphocytes. FISH results indicated that the majority (at least 18 out of the 26 cases) of the chromosome 12 breakpoints in these tumor cell lines were found to be clustering within the 175 kb DNA interval between RM99 and RM133, indicating this interval to constitute the main breakpoint cluster region. FISH results obtained with Li-501/SV40 indicated that part of MAR was translocated to an apparently normal chromosome 3; a chromosome aberration overseen by applied cytogenetics. Of interest to note, finally, is the fact that the breakpoints of uterine leiomyoma cell lines LM-5.1/SV40, LM-65/SV40, and LM-608/SV40 were found to be mapping within the same cosmid clone; i.e. cosmid 27E12.

We also performed FISH experiments on eight different types of primary benign solid tumors with chromosome 12q13-q15 aberrations (Table 4). A mixture of cosmid clones 27E12 and 142H1 was used as molecular probe. In summary, the results of the FISH studies of primary tumors were consistent with those obtained for the tumor cell lines. The observation that breakpoints of each of the seven different tumor types tested were found within the same 175 kb DNA interval of MAR suggested that this interval is critically relevant to the development of these tumors and, therefore, might harbor the putative MAG locus or critical part(s) of it.

3.3. Identification of candidate genes mapping within MAR

In an attempt to identify candidate genes mapping within the 175 kb subregion of MAR between STSs RM99 and RM133, we used 3'-terminal exon trapping and genomic sequence sampling (GSS) [63]. Using the GSS approach, we obtained DNA sequence data of the termini of a 4.9 kb BamHI subfragment of cosmid 27E12, which was shown by FISH analysis to be split by the chromosome 12 aberrations in three of the uterine leiomyoma cell lines tested. A BLAST [64] search revealed that part of these sequences displayed sequence identity with a publicly available partial cDNA sequence (EMBL accession # Z31595) of the high mobility group (HMG) protein gene HMGI-C [65], which is a member of the HMG gene family [66]. In light of these observations, HMGI-C was considered a candidate MAG gene and studied in further detail.

3.4. Genomic organization of HMGI-C and rearrangements in benign solid tumors

Since only 1200 nucleotides of the HMGI-C transcript (reported size approximately 4 kb [65, 67]) were publicly available, we first determined most of the remaining nucleotide sequences of the HMGI-C transcript (GenBank, # U28749). This allowed us to subsequently establish the genomic organization of the gene. Of interest to note about the sequence data is that a CT-repeat is present in the 5'-UTR of HMGI-C and a GGGGT-pentanucleotide repeat in the 3'-UTR, which might be of regulatory relevance. Comparison of transcribed to genomic DNA sequences (GenBank, # U28750, U28751, U28752, U28753, and U28754) of the gene revealed that HMGI-C contains at least 5 exons (Fig. 2). Transcriptional orientation of the gene is directed towards the telomere of the long arm of the chromosome. Each of the first three exons encode a putative DNA binding domain (DBD), and exon 5 encodes an acidic domain, which is separated from the three DBDs by a spacer domain encoded by exon 4. The three DBD-encoding exons are positioned relatively close together and are separated by a large intron of about 140 kb from the two other exons, which in turn are separated about 11 kb from each other. Of particular interest to emphasize here is that th fiv exons are dispersed

over a genomic region of at least 160 kb, thus almost covering the entire 175 kb main MAR breakpoint cluster region described above. Results of molecular cytogenetic studies, using a mixture of cosmids 142H1 (containing exons 1-3) and 27E12 (containing exons 4 and 5) as molecular probe, clearly demonstrate that the HMGI-C gene is directly affected by the observed chromosome 12 aberrations in the majority of the tumors and tumor cell lines that were evaluated (Fig. 3; Table 4). These cytogenetic observations were independently confirmed by Southern blot analysis in the case of LM-608/SV40 (results not shown) LM-30.1/SV40 [24], and Ad-312/SV40; probes used included CH76, RM118-A, and EM26. The failure to detect the breakpoints of LM-65/SV40, LM-609/SV40, Ad-211/SV40, Ad-263/SV40, Ad-302/SV40, Li-14/SV40, and Li-538/SV40 with any of these three probes was also consistent with the FISH data establishing the relative positions of the breakpoints in MAR (cf. Fig. 3). These results made HMGI-C a prime candidate to be the postulated MAG gene.

3.5. Expression of aberrant HMGI-C transcripts in benign solid tumor cells.

In the context of follow-up studies, it was of interest to test for possible aberrant HMGI-C expression. Initial Northern blot studies revealed that transcripts of HMGI-C could not be detected in a variety of normal tissues (brain, heart, lung, liver, kidney, pancreas, placenta, skeletal muscle) tested as well as in a number of the tumor cell lines listed in Fig. 3 (data not shown). It is known that HMGI-C mRNA levels in normal differentiated tissues are very much lower than in malignant tissues [65, 67]. As a control in our Northern studies, we included hepatoma cell line Hep 3B, which is known to express relatively high levels of HMGI-C. We readily detected two major HMGI-C transcripts, approximately 3.6 and 3.2 kb in size; with the differences in molecular weight most likely due to differences in their 5'-noncoding regions. In an alternative and more sensitive approach to detect HMGI-C or 3'-aberrant HMGI-C transcripts, we have performed 3'-RACE experiments. In control experiments using a number of tissues with varying HMGI-C transcription levels (high levels in Hep 3B hepatoma cells, intermediate in Hep G2 hepatoma cells, and low in myometrium, normal fat tissue, and pseudomyxoma), we obtained 3'-RACE clones which, upon molecular cloning and nucleotide sequence analysis, appeared to represent perfect partial cDNA copies of 3'-HMGI-C mRNA sequences; no matter which of the three selected primer sets was used (see Methodology). RACE products most likely corresponding to cryptic or aberrantly spliced HMGI-C transcripts were occasionally observed; their ectopic sequences were mapped back to HMGI-C intron 3 or 4.

In similar 3'-RACE analysis of ten different primary tumors or tumor cell lines derived from lipoma, uterine leiomyoma, and pleomorphic salivary gland adenoma, we detected both constant and unique PCR products. The constant PCR products appeared to represent, in most cases, perfect partial cDNA copies of 3'-HMGI-C mRNA sequences. They most likely originated from a presumably unaffected HMGI-C allele and might be considered as internal controls. The unique PCR products of the ten tumor cell samples presented here appeared to contain ectopic sequences fused to HMGI-C sequences. In most cases, the ectopic sequences were found to be derived from the established translocation partners, thus providing independent evidence for translocation-induced rearrangements of the HMGI-C gene. Information concerning nucleotide sequences, diversion points, and chromosomal origins of the ectopic sequences of these RACE products is summarized in Table 5. It should be noted that chromosomal origins of ectopic sequences was established by CASH (Chromosome Assignment using Somatic cell Hybrids) analysis using the NIGMS Human/Rodent Somatic Hybrid Mapping Panel 2 obtained from the Coriell Cell Repositories. Chromosomal assignment was independently confirmed by additional data for cases pCH1111, pCH172, pCH174, pCH193, and pCH117, as further outlined in Table 5. Taking into account the limitations of conventional cytogenetic analysis, especially in cases with complex karyotypes, the chromosome assignments of the ectopic sequences are in good agreement with the previous cytogenetic description of the translocations.

Somewhat unexpected were the data obtained with Ad-312/SV40, as available molecular cytogenetic analysis had indicated its chromosome 12 breakpoint to map far outside the HMGI-C gene; over 1 Mb [42]. The ectopic sequences appeared to originate from chromosome 1 (more precisely from a segment within M.I.T. YAC contig WC-511, which is partially mapping at 1p22), the established translocation partner (Fig. 2). Further molecular analysis is required to precisely define the effect on functional expression of the aberrant HMGI-C gene in this particular case. Of further interest to note here, is that the sequences coming from chromosome 1 apparently remove the GGGGT repeat observed in the 3'-UTR region of HMGI-C, as this repeat is not present in the RACE product. In contrast, in primary uterine leiomyoma LM-#58 (t(8;12)(q24;q14-q15)), which was shown to have its breakpoint also in the 3'-UTR, this repeat appeared to be present in the RACE product. Therefore, removal of this repeat is most probably not critical for tumor development. The results with primary tumor LM-#168.1, in which the X chromosome is the cytogenetically assigned translocation partner, revealed that the ectopic sequences were derived from chromosome 14; the preferential translocation partner in leiomyoma. It is possible that involvement of chromosome 14 cannot be detected by standard karyotyping in this particular case, as turned out to be the case for Li-501/SV40. In primary lipoma Li-#294 (t(8;12)(q22;q14)), two alternative ectopic sequences were detected. Additional CASH analysis using a hybrid cell mapping panel for regional localization of probes t human chromosome 8 [68] revealed that these were both derived from chromosome 8q22-qter (Table 5). It is very well possible that these RACE products correspond to alternatively spliced transcripts. Finally, in four of the

cases (Table 5, cases pCH114, pCH110, pCH109, pCH116), the RACE products appeared to correspond to cryptic or aberrantly spliced HMGI-C transcripts, as the corresponding ectopic sequences were found to be derived from either HMGI-C intron 3 or 4. Such RACE products have also been observed in th control experiments described above. In conclusion, the detection of aberrant HMGI-C transcripts in the tumor cells provides additional strong support of HMGI-C being consistently rearranged by the various chromosome 12 aberrations. It should be noted that the aberrant HMGI-C transcripts in the various cases should be characterized in full length before any final conclusion can be drawn about biological implications.

A first and preliminary evaluation of isolated ectopic sequences revealed in phase open reading frames of variable length. In the case of primary tumor LM-#25, for instance, already the second codon in the ectopic sequences appeared to be a stop codon (Table 5). A note of caution is appropriate here, as sequence data have been obtained only for clones that were produced via two rounds of extensive (probably mutations inducing) PCR. For Li-501/SV40, it is of interest to note that, in Northern blot analysis, the isolated ectopic sequences detected a transcript of over 10 kb in a variety of tissues, including heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle, but not in brain (data not shown). As chromosome 3 is the preferred partner in the chromosome 12q13-q15 translocations in lipomas and the chromosome 3 breakpoints of various lipomas were found to be spanned by YAC clone CEPH192B10, the detected transcript might correspond to a putative lipoma-preferred partner gene (LPP).

4. Discussion

In ANNEX 1 it was demonstrated that the chromosome 12q13-q15 breakpoints of lipoma, pleomorphic salivary gland adenoma, and uterine leiomyoma, irrespective of their cytogenetic assignments in the past to segment q13, q14, or q15 of chromosome 12, all cluster within the 1.7 Mb DNA interval designated MAR. In support of the claimed clustering of breakpoints is a recent study by Schoenberg Fejzo et al. [14], identifying a CEPH mega-YAC spanning the chromosome 12 translocation breakpoints in two of the three tumor types. In the present study, we have conclusively demonstrated by FISH analysis that chromosome 12 breakpoints of seven different solid tumor types are clustering within a relatively small (175 kb) segment of MAR. For some tumor cell lines, Southern blot data were obtained and these were always in support of the FISH results. From all these observations, we conclude that this segment of MAR constitutes a major target area for the chromosome 12 aberrations in these tumors and that it is likely to represent the postulated MAG locus: the multi-tumor aberrant growth locus that might be considered as common denominator in these tumors.

Within the 175 kb MAR segment, we have identified the HMGI-C gene and determined characteristics of its genomic organization. Structurally, the HMGI-C-encoded phosphoprotein consists of three putative DNA binding domains, a spacer region, and an acidic carboxy-terminal domain, and contains potential sites of phosphorylation for both casein kinase II and p34/cdc2 [65, 67]. We have provided strong evidence that HMGI-C is a prime candidate target gene involved in the various tumor types studied here. In FISH studies, the breakpoints of 29 out of 33 primary tumors were found to be mapping between two highly informative cosmids 142H1 and 27E12; the first one containing the three DBD-encoding exons and the second one, the remaining exons that code for the two other domains. Therefore, the majority of the breakpoints map within the gene, most of them probably within the 140 kb intron (intron 3), which is also in line with FISH results obtained with the 26 tumor cell lines that were evaluated. It should also be noted that the 5'-end of the HMGI-C gene is not yet fully characterized. As HMGI(Y), another member of this gene family, is known to possess various alternative first exons [69], the size of the HMGI-C gene might be larger than yet assumed. Further support that HMGI-C is affected by the chromosome 12 aberrations can be deduced from the results of the 3'-RACE experiments. Aberrant HMGI-C transcripts were detected in tumor cells, consisting of transcribed HMGI-C sequences fused to newly acquired sequences, in most cases clearly originating from the chromosomes that were cytogenetically identified as the translocation partners. It is noteworthy that many chromosomes have been found as translocation partner in the tumors studied. This observed heterogeneity in the reciprocal breakpoint regions involved in these translocations resembles that of a variety of hematological malignancies with chromosome 11q23 rearrangements involving the MLL gene [70], the translational product of which carries an amino-terminal motif related to the DNA-binding motifs of HMGI proteins.

An intriguing issue pertains to the effect of the chromosome 12 aberrations on expression of the HMGI-C gene and the direct physiological implications. Some functional characteristics of HMGI-C are known or may be deduced speculatively from studies of other family members. As it binds in the minor groove of DNA, it has been suggested that HMGI-C may play a role in organising satellite chromatin or act as a transcription factor [71, 72]. Studies on HMGI(Y), which is the member most closely related to HMGI-C, have suggested that HMGI(Y) may function as a promoter-specific accessory factor for NF-κ B transcriptional activity [73]. HMGI(Y) has also been shown to stimulate or inhibit DNA binding of distinct transcriptional factor ATF-2 isoforms [74]. Both studies indicate that the protein may simply constitute a structural component of the transcriptional apparatus functioning in promoter/enhancer contexts. In a recent report on high mobility group protein 1 (HMG1), yet another member of the HMG gene family with a similar domain structur as HMGI-C and acting as a quasi-transcription factor in gen transcription, a truncated HMG1 prot in lacking the acidic

carboxy-terminal region was shown to inhibit gene transcription [75]. It was put forward that the acidic terminus of the HMG1 molecule is essential for the enhancement of gene expression in addition to elimination of the repression caused by the DNA binding. As most of the chromosome 12 breakpoints seem to occur in the 140 kb intron, separation of the DBDs from the acidic carboxy-terminal domain seems to occur frequently. In case the acidic domain in HMGI-C has a similar function as the one in HGMI(Y), the result of the chromosome 12 aberrations is likely to affect gene expression. Finally, it should be noted that the fate of th sequences encoding the acidic carboxy-terminal region is not yet known.

As HMGI-C is the first member of the HMG gene family that might be implicated in the development of benign tumors, the question arises as to whether other members of this family could also be involved. The HMG protein family consists of three subfamilies: i) the HMG1 and 2 type proteins, which have been found to enhance transcription in vitro and may well be members of a much larger class of regulators with HMG boxes; ii) the random-coil proteins HMG14 and 17 with an as yet unknown function; iii) the HMGI-type proteins, which bind to the minor groove and include HMGI-C, HMGI, and HMGI-Y; the latter two are encoded by the same gene. It is of interest to note that published mapping positions of members of the HMG family coincide with published chromosome breakpoints of benign solid tumors such as those studied here. The HMGI(Y) gene, for instance, has been mapped to human chromosome 6p21 [69], which is known to be involved in recurrent translocations observed in uterine leiomyoma, lipoma, and pleomorphic salivary gland adenoma [76]. As listed in the Human Genome Data Base, not all known members of the HMG family have been chromosomally assigned yet, although for some of them a relatively precise mapping position has been established. For instance, HMG17 to chromosome 1p36.1-p35, HMG1L to 13q12, and HMG14 to 21q22.3; all chromosome segments in which chromosome breakpoints of the tumor types studied here have been reported [76]. Whether HMGI(Y) or any other of these HMG members are indeed affected in other subgroups of these tumors remains to be established. Of interest to mention, furthermore, are syndromes such as Bannayan-Zonana (McKusick #153480), Proteus (McKusick #176920), and Cowden (McKusick #158350); the latter syndrome is also called multiple hamartoma syndrome. In 60% of the individuals with congenital Bannayan-Zonana syndrome, a familial macrocephaly with mesodermal hamartomas, discrete lipomas and hemangiomas were found [70].

Finally, one aspect of our results should not escape attention. All the tumors that were evaluated in this study were of mesenchymal origin or contained mesenchymal components. It would be of great interest to find out whether the observed involvement of HMGI-C is mesenchyme-specific or may be found also in tumors of non-mesenchymal origin. The various DNA clones we describe here are valuable resources to address this important issue and should facilitate studies to conclusively implicate the HMGI-C gene in tumorigenesis.

EXAMPLE 3

Hybrid HMGI-C in lipoma cells.

cDNA clones of the chromosome 3-derived lipoma-preferred partner gene LPP (>50 kb) were isolated and the nucleotide sequence thereof established. Data of a composite cDNA are shown in Fig. 4. An open reading frame for a protein (612 amino acids (aa)) with amino acid sequence similarity (over 50%) to zyxin of chicken was identified. Zyxin is a member of the LIM protein family, whose members all possess so-called LIM domains [78]. LIM domains are cysteine-rich, zinc-binding protein sequences that are found in a growing number of proteins with divers functions, including transcription regulators, proto-oncogene products, and adhesion plaque constituents. Many of the LIM family members have been postulated to play a role in cell signalling and control of cell fate during development. Recently, it was demonstrated that LIM domains are modular protein-binding interfaces [79]. Like zyxin, which is present at sites of cell adhesion to the extracellular matrix and to other cells, the deduced LPP-encoded protein (Fig. 6) possesses three LIM domains and lacks classical DNA-binding homeodomains.

In 3'-RACE analysis of Li-501/SV40, a HMGI-C containing fusion transcript was identified from which a hybrid protein (324 aa) could be predicted and which was subsequently predicted to consist of the three DBDs (83 aa) of HMGI-C and, carboxy-terminally of these, the three LIM domains (241 aa) encoded by LPP. In PCR analysis using approriate nested amplimer sets similar HMGI-C/LPP hybrid transcripts were detected in various primary lipomas and lipoma cell lines carrying a t(3;12) and also in a cytogenetically normal lipoma. These data reveal that the cytogenetically detectable and also the hidden t(3;12) translocations in lipomas seem to result consistently in the in-phase fusion of the DNA-binding molecules of HMGI-C to the presumptive modular protein-binding interfaces of the LPP-encoded protein, thereby replacing the acidic domain of HMGI-C by LIM domains. Consequently, these protein-binding interfaces are most likely presented in the nuclear environment of these lipoma cells, where they might affect gene expression, possibly leading to aberrant growth control. Out of the large variety of benign mesenchymal tumors with chromosome 12q13-q15 aberrations, this is the first example of a chromosome translocation partner contributing recurrently and consistently to the formation of a well-defined tumor-associated HMGI-C fusion protein.

Figure 5 shows the cDNA sequence of the complete isolated LPP gene.

EXAMPLE 4

Diagnostic test for lipoma

A biopsy of a patient having a lipoma was taken. From the material thus obtained total RNA was extracted using the standard TRIZOL™ LS protocol from GIBCO/BRL as described in the manual of the manufacturer. This total RNA was used to prepare the first strand of cDNA using reverse transcriptase (GIBCO/BRL) and an oligo dT(17) primer containing an attached short additional nucleotide stretch. The sequence of the primer used is as described in Example 2, under point 2.5.. RNase H was subsequently used to remove the RNA from the synthesized DNA/RNA hybrid molecule. PCR was performed using a gene specific primer (Example 2, point 2.5.) and a primer complementary to the attached short additional nucleotide stretch. The thus obtained PCR product was analysed by gel electroforesis. Fusion constructs were detected by comparing them with the background bands of normal cells of the same individual.

In an additional experiment a second round of hemi-nested PCR was performed using one internal primer and the primer complementary to the short nucleotide stretch. The sensitivity of the test was thus significantly improved.

Figure 8 shows a typical gel.

TABLE I

ANALYSIS OF YAC CLONES

CEPH-Code	Size (kb)	Landmark left	#	Landmark right	#	Chimeric
183F3	715			[RM10]		YES (L + R)
70E1	450	RM29	U27125			ND
95F1	390			RM30	U29054	ND
201H7	320	RM13	U29051	RM14	U29053	ND
186G12	320					ND
354B6	280					YES (R)
126G8	410					ND
258F11	415	RM4	U29052			ND
320F6	290	RM5	U29050	RM21	U29047	ND
234G11	475	RM7	U29046			ND
375H5	290					ND
262E10	510	[RM15]		RM16	U29048	YES (L)
181C8	470			RM26	U29045	ND
107D1	345	RM31	U29043			ND
499C5	320	RM44	U29044	RM46	U29037	ND
340B6	285					ND
532C12	400	RM45	U29041			ND
138C5	510	[RM59]		RM65	U29042	YES (L)
145F2	490	RM60	U29030	RM66	U29040	ND
106E8	340	RM57	U29033	RM63	U29038	ND
55G1	365	RM56	U29031	RM62	U29039	ND
103G7	370	RM85	U29025	RM80	U29036	ND
295B10	295	RM77	U29035	RM81	U29026	ND
338C2	200	RM78	U29034	RM82	U29029	ND
391C12	160	[RM79]		RM83	U29027	YES (L)
476A11	225	[RM87]		RM84	U29032	YES (L)
138F3	460	RM90	U29028	RM91	U29019	ND
226E7	500	RM48	U29024	RM54	U29015	ND
499E9	375	RM51	U29016			YES (R)
312F10	580	[RM50]		RM69	U29021	YES (L)
825G7	950					ND
34B5	315	RM88	U29020	RM89	U29013	ND
94A7	610					YES (R)
305B2	660					YES (L)
379H1	280	RM104	U29014	RM105	U29009	ND
444E6	350	RM92	U29017	RM93	U29010	ND
446H3	370	RM94	U29011	RM95	U29018	ND
403B12	380					ND
261E5	500	RM102	U29012	RM103	U26589	ND
78B11	425					ND
921B9	1670					ND
939H2	1750					ND
188H7	360					ND
142F4	390					ND
404E12	360					ND
164A3	375					ND
244B12	415	RM106	U29007	RM107	U29008	ND
275H4	345	RM108	U29004	RM109	U29005	ND
320F9	370					ND
51F8	450					ND
242A2	160	CH1	U29006			ND

TABLE I (ontinued)

ANALYSIS OF YAC CLONES

253H1	400					ND
303F11	320					ND
322C8	410			CH2	U29003	ND
208G12	370	RM96	U29002	RM97	U27135	ND
341C1	270	RM98	U26647	RM99	U27130	ND
354F1	270					ND
452E1	270	CH5	U27136			ND
41A2	310					ND
934D2	1370					ND
944E8	1290			CH8	U28792	ND
2G11	350					ND
755D7	1390					YES (L)
365A12	370					ND
803C2	1080					ND
210C1	395	RM70	U28998	RM86	U27133	ND
433C8	360	RM73	U29000	RM76	U27132	ND
402A7	500	RM41	U28994	[RM42]		YES (R)
227E8	465	RM53	U27134	RM55	U28996	ND
329F9	275	RM72	U28793	RM75	U28997	ND
261E6	395	[RM71]		RM74	U28995	YES (L)
348F2	370			[RM136]		YES (R)
6F3	320	RM35	U27140	RM36	U27141	
59F12	430	RM34	U28794	RM33	U27131	
265H3	300			RM40	U28999	

YAC clones were isolated from CEPH YAC libraries as described in Materials and Methods. ND: not detected by methods used. Landmarks not mapping within the 6 Mb contig have been bracketed. GenBank accession numbers are given (#).

TABLE II

PCR Primers

STS name (STS 12-)	Nucleotide sequence 5'-3'	Product size (bp)	T_{am} (°C)
CH1	TGGGACTAACGGATTTTCAA TGTGGTTCATTCATGCATTA	213	58
CH2	TCCATCATCATCTCAAAACA CTCTACCAAATGGAATAAACAG	145	58
CH5	GCAGCTCAGGCTCCTTCCCA TGGCTTCCTGAAACGCGAGA	143	58
CH8	TCTCCACTGCTTCCATTCAC ACACAAAACCACTGGGGTCT	147	58
CH9	CAGCTTTGGAATCAGTGAGG CCTGGGGAAGAGGAGTAAAG	262	58
RM1	GAGCTTCCTATCTCATCC ATGCTTGTGTGTGAGTGG	308	60
RM4	TTTGCTAAGCTAGGTGCC AGCTTCAAGACCCATGAG	236	60
RM5	CAGTTCTGAGACTGCTTG TAATAGCAGGGACTCAGC	324	60
RM7	CTTGTCTCATTCTTTTAAAGGG CACCCCTTTTTAGATCCTAC	538	58
RM13	GAATGTTCATCACAGTGCTG AATGTGAGGTTCTGCTGAAG	±500	58
RM14	TTCTCATGGGGTAAGGACAG AAAGCTGCTTATATAGGGAATC	158	58
RM16	CCTTGGCTTAGATATGATACAC GCTCTTCAGAAATATCCTATGG	252	58
RM21	CCTTAGCAGTTGCTTGTCTG TCGTCACAGGACATAGTCAC	290	58
RM26	TCTATGGTATGTTATACAAGATG CAGTGAGATCCTGTCTCTA	102	58
RM31	TCTGTGATGTTTTAAGCCACTTAG AATTCTGTGTCCCTGCCACC	239	56
RM33	ATTCTTCCTCACCTCCCACC AATCTGCAGAGAGGTCCAGC	±600	60
RM34	AATTCTCCATCTGGGCCTGG GAACGCTAAGCATGTGGGAG	±600	60
RM36	CTCCAACCATGGTCCAAAAC GACCTCCAGTGGCTCTTTAG	296	60
RM46	ACCATCAGATCTGGCACTGA TTACATTGGAGCTGTCATGC	241	57
RM48	TCCAGGACATCCTGAAAATG AGTATCCTGCACTTCTGCAG	391	58
RM51	GATGAACTCTGAGGTGCCTTC TCAAACCCAGCTTTGACTCC	311	60
RM53	GTCTTCAAAACGCTTTCCTG TGGTTTGCATAATGGTGATG	333	60

TABLE II (continued)

PCR Primers

STS name (STS 12-)	Nucleotide sequence 5'-3'	Product size (bp)	T_{ann} (°C)
RM60	TACACTACTCTGCAGCACAC TCTGAGTCAATCACATGTCC	94	58
RM69	CTCCCCAGATGATCTCTTTC CGGTAGGAAATAAAGGAGAG	236	58
RM72	TATTTACTAGCTGGCCTTGG CATCTCAGGCACACACAATG	101	62
RM76	ATTCAGAGAAGTGGCCAAGT GGGATAGGTCTTCTGCAATC	496	58
RM85	TCCAACAATACTGAGTGACC TCCATTTCACTGTAGCACTG	435	58
RM86	GTAATCAACCATTCCCCTGA AAAATAGCTGGTATGGTGGC	203	56
RM90	ACTGCTCTAGTTTTCAAGGA AATTTACCTGACAGTTTCCT	257	58
RM93	GCATTTGACGTCCAATATTG ATTCCATTGGCTAACACAAG	347	60
RM98	GCAAAACTTTGACTGAAACG CACAGAGTATCGCACTGCAT	356	58
RM99	AAGAGATTTCCCATGTTGTG CTAGTGCCTTCACAAGAACC	240	58
RM103	AATTCTTGAGGGGTTCACTG TCCACACTGAGAGCTTTTCA	199	60
RM108	GTGGTTCTGTACAGCAGTGG TGAGAAAATGTCTGCCAAAT	439	60
RM110	GCTCTACCAGGCATACAGTG ATTCCTAGCATCTTTTCACG	328	58
RM111	ATATGCATTAGGCTCAACCC ATCCCACAGGTCAACATGAC	312	58
RM130	ATCCTTACATTTCCAGTGGCATTCA CCCAGAAGACCCACATTCCTCAT	336	58
RM131	TTTTAAGTTTCTCCAGGGAGGAGAC AATAGGCTCTTTGGAAAGCTGGAGT	226	58
RM132	TCTCAGCTTAATCCAAGAAGGACTTC GGCATATTCCTCAACAATTTATGCTT	376	58
RM133	TGGAGAAGCTATGGTGCTTCCTATG TGACAAATAGGTGAGGGAAAGTTGTTAT	225	58
EST01096	TCACACGCTGAATCAATCTT CAGCAGCTGATACAAGCTTT	188	58
IFNG	TGTTTTCTTTCCCGATAGGT CTGGGATGCTCTTCGACCTC	150	52
Rap1B	CCATCCAACATCTTAAATGGAC CAGCTGCAAACTCTAGGACTATT	149	58

STSs were isolated as described in Materials and Methods, or retrieved from literature for EST01096, IFNG, and Rap1B.

Table 3

Genome Data Base accession numbers (D-numbers) of the various sequences indicated in Figure 1.

Genome Data Base

pcr	locus_symbol	pcr	pcr_gdb_id	(41 rows affected) locus	locus_gdb_id
CH1-lower/CH1-upper	D12S1484	CH1-lower/CH1-upper	G00-595-292	D12S1484	G00-595-415
CH2-lower/CH2-upper	D12S1485	CH2-lower/CH2-upper	G00-595-295	D12S1485	G00-595-416
CH5-lower/CH5-upper	D12S1486	CH5-lower/CH5-upper	G00-595-298	D12S1486	G00-595-417
CH8-lower/CH8-upper	D12S1487	CH8-lower/CH8-upper	G00-595-301	D12S1487	G00-595-418
CH9-lower/CH9-upper	D12S1488	CH9-lower/CH9-upper	G00-595-304	D12S1488	G00-595-419
EM2-lower/EM2-upper	D12S1489	EM2-lower/EM2-upper	G00-595-307	D12S1489	G00-595-420
EM3-lower/EM3-upper	D12S1490	EM3-lower/EM3-upper	G00-595-310	D12S1490	G00-595-421
EM4-lower/EM4-upper	D12S1491	EM4-lower/EM4-upper	G00-595-313	D12S1491	G00-595-422
RM13-lower/RM13-upper	D12S1492	RM13-lower/RM13-upper	G00-595-316	D12S1492	G00-595-423
RM14-lower/RM14-upper	D12S1493	RM14-lower/RM14-upper	G00-595-319	D12S1493	G00-595-424
RM16-lower/RM16-upper	D12S1494	RM16-lower/RM16-upper	G00-595-322	D12S1494	G00-595-425
RM25-lower/RM25-upper	D12S1507	RM26-lower/RM26-upper	G00-595-325	D12S1495	G00-595-426
RM26-lower/RM26-upper	D12S1495	RM-29-lower/RM29-upper	G00-595-328	D12S1496	G00-595-427
RM31-lower/RM31-upper	D12S1497	RM31-lower/RM31-upper	G00-595-331	D12S1497	G00-595-428
RM33-lower/RM33-upper	D12S1498	RM33-lower/RM33-upper	G00-595-334	D12S1498	G00-595-429
RM34-lower/RM34-upper	D12S1499	RM34-lower/RM34-upper	G00-595-337	D12S1499	G00-595-430
RM36-lower/RM36-upper	D12S1500	RM36-lower/RM36-upper	G00-595-340	D12S1500	G00-595-431
RM46-lower/RM46-upper	D12S1501	RM46-lower/RM46-upper	G00-595-343	D12S1501	G00-595-432
RM48-lower/RM48-upper	D12S1502	RM48-lower/RM48-upper	G00-595-346	D12S1502	G00-595-433
RM51-lower/RM51-upper	D12S1503	RM51-lower/RM51-upper	G00-595-349	D12S1503	G00-595-434
RM53-lower/RM53-upper	D12S1504	RM53-lower/RM53-upper	G00-595-352	D12S1504	G00-595-435
RM60-lower/RM60-upper	D12S1505	RM60-lower/RM60-upper	G00-595-355	D12S1505	G00-595-436
RM69-lower/RM69-upper	D12S1506	RM69-lower/RM69-upper	G00-595-358	D12S1506	G00-595-437
RM72-lower/RM72-upper	D12S1508	RM25-lower/RM25-upper	G00-595-361	D12S1507	G00-595-438
RM76-lower/RM76-upper	D12S1509	RM72-lower/RM72-upper	G00-595-364	D12S1508	G00-595-439
RM85-lower/RM85-upper	D12S1510	RM76-lower/RM76-upper	G00-595-367	D12S1509	G00-595-440
RM86-lower/RM86-upper	D12S1511	RM85-lower/RM85-upper	G00-595-370	D12S1510	G00-595-44[illegible]
RM90-lower/RM90-upper	D12S1512	RM86-lower/RM86-upper	G00-595-373	D12S1511	G00-595-442
RM93-lower/RM93-upper	D12S1513	RM90-lower/RM90-upper	G00-595-376	D12S1512	G00-595-443
RM98-lower/RM98-upper	D12S1514	RM93-lower/RM93-upper	G00-595-379	D12S1513	G00-595-444
RM99-lower/RM99-upper	D12S1515	RM98-lower/RM98-upper	G00-595-382	D12S1514	G00-595-445
RM-29-lower/RM29-upper	D12S1496	RM99-lower/RM99-upper	G00-595-385	D12S1515	G00-595-446
RM103-lower/RM103-upper	D12S1516	RM103-lower/RM103-upper	G00-595-388	D12S1516	G00-595-447
RM108-lower/RM108-upper	D12S1517	RM108-lower/RM108-upper	G00-595-391	D12S1517	G00-595-448
RM110-lower/RM110-upper	D12S1518	RM110-lower/RM110-upper	G00-595-394	D12S1518	G00-595-449
RM111-lower/RM111-upper	D12S1519	RM111-lower/RM111-upper	G00-595-397	D12S1519	G00-595-450
RM121-lower/RM121-upper	D12S1520	RM121-lower/RM121-upper	G00-595-400	D12S1520	G00-595-451
RM130-lower/RM130-upper	D12S1521	RM130-lower/RM130-upper	G00-595-403	D12S1521	G00-595-452
RM131-lower/RM131-upper	D12S1522	RM131-lower/RM131-upper	G00-595-406	D12S1522	G00-595-453
RM132-lower/RM132-upper	D12S1523	RM132-lower/RM132-upper	G00-595-409	D12S1523	G00-595-454
RM133-lower/RM133-upper	D12S1524	RM133-lower/RM133-upper	G00-595-412	D12S1524	G00-595-455

TABLE 4

FISH mapping of chromosome 12 breakpoints in primary benign solid tumors to a subregion of MAR		
Tumor type	Breakpoint within MAR	Fraction of tumors with breakpoints within main breakpoint cluster region*
Lipoma	6/6	6/6
Pleomorphic salivary gland adenoma	7/7	5/7
Uterine leiomyoma	7/8	7/8
Hamartoma of the breast	1/1	1/1
Fibroadenoma of the breast	1/1	1/1
Hamartoma of the lung	8/9	8/9
Angiomyxoma	1/1	1/1

* Tumor samples were collected and analyzed at the histopathology and cytogenetics facilities of the University of Bremen. A mixture of cosmid clones 27E12 and 142H1 was used as molecular probe in FISH analysis.

Table 5-1

Clone	Tumor/Cell Line	Diversion Point	Nuc. Sequences (10b)		Chrom. RACE	sources cytogen.		poly(A) signal	#A's	primer-set
		exon/intron 3 (DBD3)	TAGGAAATGG	GTGAGTAATA						
pCH108	L1-14/SV40	after DBD3	TAGGAAATGG	AATACTCTGG	12	?	3q27	AGTAAA	26	?
pCH113	L1-538/SV40	after DBD3	TAGGAAATGG	AATACTCTGG	12	?	3q27	AGTAAA	26	?
pCH234	#2528-90	after DBD3	TAGGAAATGG	AATACTCTGG	12	?		(AGTAAA)	17	
pCH259	#2344-94	after DBD3	TAGGAAATGG	AATACTCTGG	12	?		?	?	
pCH260	#2344-94	after DBD3	TAGGAAATGG	AATACTCTGG	12	?		?	?	
pCH148	#192	after DBD3	TAGGAAATGG	CCTACTATTG	12	N.T.[1]	12	AATAAA	18	---
pCH245	#568-92	after DBD3	TAGGAAATGG	CCTACTATTG	12			AATAAA	17	
pCH247	#568-92	after DBD3	TAGGAAATGG	CCTACTATTG	12			AATAAA	53	
pCH261	#2344-94	after DBD3	TAGGAAATGG	CCTACTATTG	12			AATAAA	27	
pCH212	#1321-89 ?	after DBD3	TAGGAAATGG	GGAAGTGTGA	12			AATAAA	16	
pCH213	#1321-89 ?	after DBD3	TAGGAAATGG	GGAAGTGTGA	12			?	?	
pCH228	#1321-89	after DBD3	TAGGAAATGG	GGAAGTGTGA	12			N.D.	13	
pCH216	#2100-89	after DBD3	TAGGAAATGG	AACACAGGAC	12			AATAAA	12	
pCH229	#2100-89	after DBD3	TAGGAAATGG	AACACAGGAC	12			AATAAA	15	
pCH232	#2100-89	after DBD3	TAGGAAATGG	AACACAGGAC	12			AATAAA	25	
pCH177	#367	after DBD3	TAGGAAATGG	GTTTAATATT	12	3	2p21	AATAAA	29	177
pCH191	#25 ?	after DBD3	TAGGAAATGG	GTTTAATATT	12	3	?	AATAAA	49	(177)
pCH210	#837-88	after DBD3	TAGGAAATGG	AAGAAGGCAG	12			AATAAA	22	
pCH211	#837-88	after DBD3	TAGGAAATGG	AAGAAGGCAG	12			AATAAA	22	
pCH230	#2100-89	after DBD3	TAGGAAATGG	TAGGAGGTAG	12			AATAAA	27	
pCH233	#2100-89	after DBD3	TAGGAAATGG	TAGGAGGTAG	12			AATAAA	18	
pCH111	L1-501/SV40	after DBD3	TAGGAAATGG	GGTGGCCATT	12	3	3q27	AATAAA	33	111-AB
pCH112	L1-501/SV40	after DBD3	TAGGAAATGG	GGTGGCCATT	12	3	3q27	AATAAA	33	111-AB
pCH114	L1-538/SV40	after DBD3	TAGGAAATGG	GACAATCTAC	12	12	3q27	(CATAAA)	24	115-AB
pCH115	L1-538/SV40	after DBD3	TAGGAAATGG	GACAATCTAC	12	12	3q27	(CATAAA)	24	115-AB
pCH147	#192	after DBD3	TAGGAAATGG	GTACAGAAGA	12	9	12	AATAAA	23	147
pCH153	#203	after DBD3	TAGGAAATGG	GGGCATTCAG	12	N.T.[1]	4p13	AATAAA	22	---
pCH169	#294	after DBD3	TAGGAAATGG	GCAGTCTGTA	12	?	8q22	AATAAA	26	169
pCH172	#294	after DBD3	TAGGAAATGG	TCTGTATCCT	12	8q22-qter	8q22	AATAAA	24	172-AB
pCH173	#294	after DBD3	TAGGAAATGG	ACACACTTCC	12	?	8q22	AATAAA	12	173-AB
pCH174	#294	after DBD3	TAGGAAATGG	ATATTATGGA	12	8q22	8q22	AATAAA	15	174-AB
pCH110	LM-30.1/SV40	after DBD3	TAGGAAATGG	GAGGAGTTTT	12	12	14	AATAAA	17	110-AB
pCH164	Myo168.1	after DBD3	TAGGAAATGG	TAACACAGGA	12	?	X	(GATAAA)	30	164-AB
pCH165	Myo168.1	after DBD3	TAGGAAATGG	TTACCTGCTG	12	14	X	(AATAAC)	30	165-AB
pCH168	Myo196.4	after DBD3	TAGGAAATGG	GCTGGAGTGC	12	12	14	(CATAAA)	23	168
pCH209	#837-88 ?	after DBD3	TAGGAAATGG	GTCTCCTCCC	12			AATAAA	19	
pCH217	#2100-89 ?	after DBD3	TAGGAAATGG	TTTNTCTCTT	12			ATTAAA	22	
pCH219	#2100-89 ?	after DBD3	TAGGAAATGG	AGTCCAAGAA	12			(CATAAA)	17	
pCH220	#3391-90	after DBD3	TAGGAAATGG	CCAAACTCTG	12			AATAAA	28	
pCH223	#CG592	after DBD3	TAGGAAATGG	CTCCAGAAAC	12			AATAAA	24	
pCH226	#3100-88	after DBD3	TAGGAAATGG	AACTTCTTGA	12			?	?	
pCH231	#2100-89	after DBD3	TAGGAAATGG	GAATGTCAGA	12			AATAAA	24	
pCH246	#568-92	after DBD3	TAGGAAATGG	CCTGGAAGCT	12			AATAAA	?	
pCH248	#568-92	after DBD3	TAGGAAATGG	ATGGAGTCTC	12			AATAAA	40	
pCH249	#2617-93	after DBD3	TAGGAAATGG	ATGGAGTCTC	12			AATAAA	19	
pCH251	#2617-93	after DBD3	TAGGAAATGG	ATGGAGTCTC	12			AATAAA	19	
pCH250	#2617-93	after DBD3	TAGGAAATGG	TTCCAGATAC	12			N.D.	13	
pCH252	#2617-93	after DBD3	TAGGAAATGG	TTCCAGATAC	12			N.D.	13	

Table 5-2

Clone	Tumor/Cell Line	Diversion Point	Nuc. Sequences (10b)		Chrom. sources RACE	cytogen.		poly(A) signal	#A's	primer-set
		exon/intron 4 (SPACER)	GCCTGCTCAG	GTAAGACATA						
pCH109	LM30.1/SV40	after spacer	GCCTGCTCAG	GTCAATGTTG	12	12	14	AATAAA	17	109-AB
pCH238	#2778-93	after spacer	GCCTGCTCAG	GTCAATGTTG	12	12	14	AATAAA	17	109-AB
pCH244	#2162-91	after spacer	GCCTGCTCAG	GTCAATGTTG	12	12	14	AATAAA	17	109-AB
pCH254	#2776-93	after spacer	GCCTGCTCAG	GTCAATGTTG	12	12	14	AATAAA	17	109-AB
pCH203	#2528-90	after spacer	GCCTGCTCAG	TCCTGGTACC	12	(NF1")		N.D.	19	
pCH199	#CG575	after spacer	GCCTGCTCAG	TCCTGGTACC	12	(NF1")	12	(AATAAA)	15	
pCH222	#CG575	after spacer	GCCTGCTCAG	TCCTGGTACC	12	(NF1")	12	N.D.	16	
pCH206	#CG575	after spacer	GCCTGCTCAG	TCCTGGTACC	12	(NF1")	12	(AATAAA)	16	
pCH175	#275	after spacer	GCCTGCTCAG	TCTTTCAGAT	12	?	1	AATAAA	15	175-AB
pCH237	#2617-93	after spacer	GCCTGCTCAG	TCTTTCAGAT	12	?		AATAAA	16	
pCH207	#2540-87	after spacer	GCCTGCTCAG	AATTACCTCT	12		12	AATAAA	18	
pCH239	#2344-94	after spacer	GCCTGCTCAG	AATTACCTCT	12			AATAAA	18	
pCH116	Li-14/SV40	after spacer	GCCTGCTCAG	GACTGACTCA	12	12	3	(AATAGA)	17	116-AB
pCH184	Myo163.1	after spacer	GCCTGCTCAG	TATTCCTGAA	12	12	14	AATAAA	19	184-AB
pCH208	#2540-87	after spacer	GCCTGCTCAG	GTCAATGTTG	12		12	AATAAA	47	
pCH224	#2540-87	after spacer	GCCTGCTCAG	AATTACCTCT	12		12	AATAAA	15	
pCH201	#837-88	after spacer	GCCTGCTCAG	AATTACCTCT	12			AATAAA	15	
pCH236	#2162-91	after spacer	GCCTGCTCAG	GCTTTTTCAA	12			AATAAA	28	
pCH243	#2162-91	after spacer	GCCTGCTCAG	GTTAAGAAAC	12			?	?	
pCH227	#183-89	after spacer	GCCTGCTCAG	GNCTGACTAC	12		12	(AATAGA)	14	
		3'-untranslated region	(various positions)							
pCH193	#58	within 3'-UTR	TATCCTTTCA	AAGTCAAGAG	12	8q22-qter	8q24	AATAAA	23	(195-AB)
pCH194	#58	within 3'-UTR	TATCCTTTCA	AAGTCAAGAG	12	8q22-qter	8q24	AATAAA	34	(195-AB)
pCH195	#192	within 3'-UTR	TATCCTTTCA	AAGTCAAGAG	12	8	12	AATAAA	23	195-AB
pCH196	#192	within 3'-UTR	TATCCTTTCA	AAGTCAAGAG	12	8	12	AATAAA	>17	195-AB
pCH189	Myo192.1	within 3'-UTR	TCTTTCCACT	del CCCTGT	12	12				
			ATACCACTTA	TTTTAAAACA	12	N.T.[1]	2/3	AATAAA	23	---
pCH117	Ad-312/SV40	within 3'-UTR	TTGCCATGGT	AATCTGAAAT	12	1p22	1p22	?	?	117-AB
pCH253	#2617-93	within 3'-UTR	CACTTTCATC	ATATGGCAAG	12			N.D.		
pCH264	#568-92	within 3'-UTR	ATAAGGACTA	TCAGGCATCA	12			(AGTAAA)	19	
pCH270	#2528-90	within 3'-UTR	NCTTGTNAGC	TAGAGATTAG	12			N.D.	4	

N.T.: NOT TESTABLE:

N.T.[1]: LENGTH OF ECTOPIC SEQUENCE DOES NOT ALLOW DEVELOPMENT OF PRIMER-SET
N.T.[2]: ECTOPIC SEQUENCE IS MAINLY COMPOSED OF REPETITIVE SEQUENCES

N.D.: NOT DETECTED

LEGENDS TO THE FIGURES

Figure 1

Long range physical map of a 6 Mb region on the long arm of human chromosome 12 deduced from a YAC contig consisting of 75 overlapping CEPH YAC clones and spanning the chromosome 12q breakpoints as present in a variety of benign solid tumors. The long range physical map of the composite genomic DNA covered by the YAC inserts is represented by a black solid line with the relative positions of the various restriction sites of rare cutting enzymes indicated. DNA regions in which additional cutting sites of a particular restriction enzyme might be found are indicated by arrows. Polymorphic restriction endonuclease sites are marked with asterisks. DNA markers isolated and defined by others are depicted in green. DNA markers obtained by us are shown in boxes and are labelled by an acronym (see also Table I and II). The relative positions of these DNA markers in the long range physical map are indicated and those corresponding to particular YAC ends are linked to these by a dotted line. Some of the DNA markers have been assigned to a DNA interval and this is indicated by arrows. For DNA markers in white boxes STSs have been developed and primer sets are given in Table II. For those in yellow boxes, no primer sets were developed. The DNA intervals containing RAP1B, EST01096, or IFNG are indicated. Where applicable, D number assignments are indicated. Below the long range physical map, the sizes and relative positions of the overlapping YAC clones fitting within the consensus long range restriction map are given as solid blue lines. DNA regions of YAC inserts not fitting within the consensus long range restriction map are represented by dotted blue lines. CEPH microtiter plate addresses of the YAC clones are listed. The orientation of the YAC contig on chromosome 12 is given. The relative positions of ULCR12 and MAR are indicated by red solid lines labelled by the corresponding acronyms. Accession numbers of STSs not listed in Table I: CH9 (#U27142); RM1 (#U29049); RM110 (#U29022); RM111 (#U29023); RM130 (#U27139); RM131 (#U29001); RM132 (#U27138); RM133 (#U27137). Restriction sites: B: BssHII; K: KspI (=SacII); M: MluI; N: NotI; P: PvuI; Sf: SfiI.

Figure 2

Contig of overlapping cosmids, long range restriction and STS map spanning a segment of MAR of about 445 kb. Contig elements are numbered and defined in the list below. LL12NC01-derived cosmid clones are named after their microtiter plate addresses. GenBank accession numbers (#) of the various STSs are listed below. STSs are given in abbreviated form; e.g. RM33 instead of STS 12-RM33. A 40 kb gap between STSs "K" and "O" in the cosmid contig was covered by ■ clones (clones 38 and 40) and PCR products (clones 37 and 39). The orientation of the contig on the long arm of chromosome 12 is given as well as the order of 37 STSs (indicated in boxes or labelled with encircled capital letters). The slanted lines and arrows around some of the STS symbols at the top of the figure mark the region to which the particular STS has been assigned. It should be noted that the cosmid contig is not scaled; black squares indicate STSs of cosmid ends whereas the presence of STSs corresponding to internal cosmid sequences are represented by dots. Long range restriction map: Bs: BssHII; K: KspI (=SacII); M: MluI; N: NotI; P: PvuI; Sf: SfiI. At the bottom of the figure, detailed restriction maps are shown of those regions containing exons (boxes below) of the HMGI-C gene. Non-coding sequences are represented by open boxes and coding sequences by black boxes. Estimated sizes (kb) of introns are as indicated. The relative positions of the translation initiation (ATG) and stop (TAG) codons in the HMGI-C gene as well as the putative poly-adenylation signal are indicated by arrows. Detailed restriction map: B: BamHI; E: EcoRI; H: HindIII. MAR: Multiple Aberration Region; DBD: DNA Binding Domain.

1=140A3	11=142G8	21=124D8	31=59A1	41=128A2	51=65E6
2=202A1	12=154A10	22=128A7	32=101D8	42=142H1	52=196E1
3=78F11	13=163D1	23=129F9	33=175C7	43=204A10	53=215A8

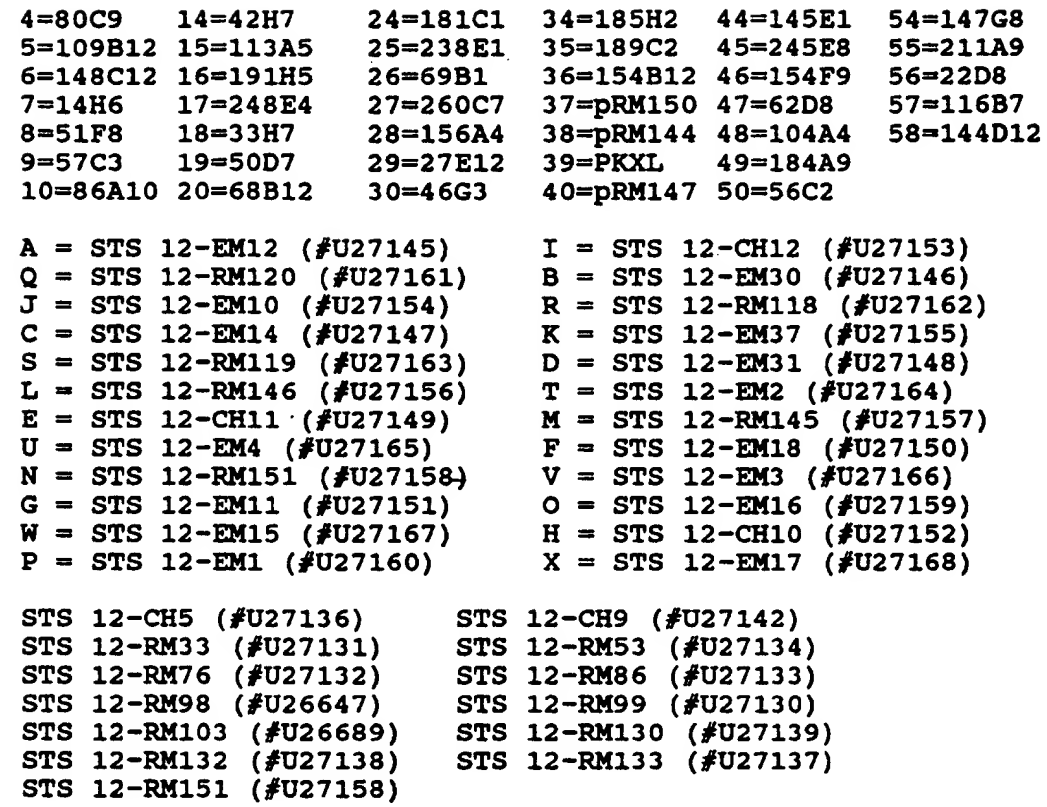


Figure 3

Schematic representation of FISH mapping data obtained for tumor cell lines with chromosome 12q13-q15 aberrations, including 8 lipoma, 10 uterine leiomyoma, and 8 pleomorphic salivary gland adenoma cell lines in consecutive experiments following our earlier FISH studies. Probes used included phage clones pRM144 (corresponding STSs: RM86 and RM130) and pRM147 (RM151), and cosmid clones 7D3 or 152F2 (RM103), 154F9 (CH9), 27E12 (EM11), 211A9 (RM33), 245E8 (RM53), 185H2 (RM76), 202A1 (RM98), 142H1 (RM99), 154B12 (RM132), and 124D8 (RM133). The DNA interval between RM33 and RM98 is estimated to be about 445 kb. Dots indicate conclusive FISH experiments that were performed on metaphase chromosomes of a particular cell line using as molecular probe, a clone containing the STS given in the box above. Solid lines indicate DNA intervals to which a breakpoint of a particular cell line was concluded to be mapping. Open triangles indicate deletions observed during FISH analysis. Open circles indicate results of FISH experiments on metaphase chromosomes of Li-501/SV40 cells with hybridization signals on a cytogenetically normal chromosome 3. The positions of chromosome 12 breakpoints of tumor cell lines mapping outside MAR are indicated by arrows. The molecularly cloned breakpoints of LM-30.1/SV40 and LM-608/SV40 are indicated by asterisks. Breakpoints in various uterine leiomyoma cell lines splitting cosmid 27E12 (EM11) are indicated by "across".

Figure 4

3'-RACE product comprising the junction between part of the HMGI-C gene and part of the LPP gene. The primers used and the junction are indicated. The cDNA synthesis was internally primed and not on the true poly(A) tail.

Figure 5

Partial cDNA sequence of the LPP gene.

Figure 6

Amino acid sequence of the LPP gene. LIM domains are boxed. The breaking point is indicated with an arrow.

Figure 7

Nucleotide sequence if HMGI-C (U28749). The transcription start site indicated as proposed by Manfioletti et al. [67] was arbitrarily chosen as a start site. The sequence contains the complete coding sequence.

Figure 8

Gel of PCR products obtained as described in Example 4.

REFERENCES

1. Sreekantaiah, C., Leong, S.L.P., Karakousis, C.P., McGee, D.L., Rappaport, W.D., Villar, H.V., Neal, D., Fleming, S., Wankel, A., Herrington, P.N., Carmona, R. and Sandberg, A.A. (1991). Cytogenetic profile of 109 lipomas. Cancer Res. **51**: 422-433.

2. Nilbert, M. and Heim, S. (1990). Uterine leiomyoma cytogenetics. Genes Chrom. Cancer **2**: 3-13.

3. Pandis, N., Heim, S., Willen, H., Bardi, G., Floderus, U.M., Mandahl, N. and Mitelman, F. (1991). Chromosome analysis of 96 uterine leiomyomas. Cancer Genet. Cytogenet. **55**: 11-18.

4. Sandros, J., Stenman, G. and Mark, J. (1990). Cytogenetic and molecular observations in human and experimental salivary gland tumours. Cancer Genet. Cytogenet. **44**: 153-167.

5. Bullerdiek, J., Wobst, G., Meyer-Bolte, K., Chilla, R., Haubrich, J., Thode, B. and Bartnitzke, S. (1993). Cytogenetic subtyping of 220 salivary gland pleomorphic adenomas: correlation to occurrence, histological subtype, and in vitro cellular behavior. Cancer Genet. Cytogenet. **65**: 27-31.

6. Walter, T.A., Xuan Fan, S., Medchill, M.T., Berger, C.S., Decker, H-J.H. and Sandberg, A.A. (1989). Inv(12)(p11.2q13) in an endometrial polyp. Cancer Genet. Cytogenet. **41**: 99-103.

7. Vanni, R., Dal Cin, P., Marras, S., Moerman, P., Andrtia, A., Valdes, E., Deprest, J., and Van den Berghe, H., (1993). Endometrial polyp: Another benign tumor characterized by 12q13-q15 changes. Cancer Genet. Cytogenet. **68**: 32-33.

8. Mandahl, N., Orndal, C., Heim, S., Willen, H., Rydholm, A., Bauer, H.C.F. and Mitelman, F. (1993). Aberrations of chromosome segment 12q13-15 characterize a subgroup of hemangiopericytomas. Cancer **71**: 3009-3013.

9. Mandahl, N., Heim, S., Arheden, K., Rydholm, A., Willen, H. and Mitelman, F. (1989). Chromosomal rearrangements in chondromatous tumors. Cancer **65**:242-248.

10. Bridge, J.A., Persons, D.L., Neff, J.R. and Bhatia, P. (1992). Clonal karyotypic aberrations in enchondroma. Cancer Detect. Prev. **16**: 215-219.

11. Hirabayashi, Y., Yoshida, M.A., Ikeuchi, T., Ishida, T., Kojima, T. Higaki, S., Machinami, R. and Tonomura, A. (1992). Chromosome rearrangements at 12q13 in two cases of chondrosarcomas. Cancer Genet. Cytogenet. **60**: 35-40.

12. Mandahl, N., Willen, H., Rydholm, A. and Mitelman, F. (1993). Rearrangement of band q13 on both chromosomes 12 in a periosteal chondroma. Genes Chrom. Cancer **6**: 121-123. 13. Dal Cin, P., Kools, P., De Jonge, I., Moerman, Ph., Van
de Ven W., Van den Berghe H. (1993a). Rearrangement of 12q14-15 in Pulmonary Chondroid Hamartoma. Genes Chrom. Cancer, **8**, 131-133.

14. Schoenberg Fejzo, M., Yoon, S.J., Montgomery, K.T., Rein, M.S., Weremowicz, S., Krauter, K.S., Dorman, T.E., Fletcher, J.A., Mao, J., Moir, D.T., Kucherlapati, R.S., and Morton, C.C. (1995). Identification of a YAC spanning the translocation breakpoints in uterine leiomyomata, pulmonary chondroid hamartoma and lipoma. Physical mapping of the 12q14-15 breakpoint region in uterine leiomyomata. Genomics **26**: 265-275.

15. Birdsal, S.H., MacLennan, K.A. and Gusterson, B.A. (1992). t(6;12)(q23;q13) and t(10;16)(q22;p11) in a phyllodes tumor of the breast. Cancer Genet. Cytogenet. **60**: 74-77.

16. Rohen, C., Bonk, U., Staats, B., Bartnizke, S. and Bullerdiek, J. (1993). Two human breast tumors with translocations involving 12q13-15 as the sole cytogenetic abnormality. Cancer Genet. Cytogenet., **69**: 68-71.

17. Jenkins, R.B., Kimmell, D.W., Moertel, C.A., Schulz, C.A., Menezes, R.M., Scheihauer, B., Kelly, P.J. and Dewald, G.W. (1989). Recurrent cytogenetic abnormalities in 80 gliomas. Cytogenet. Cell Genet. **51**: 1019.

18. Noguera, R., Llombart-Bosch, A., Lopez-Gines, C., Carda, C. and Fernandez, Cl. (1989). Giant-cell tumor of bon , stage II, displaying translocation t(12;19)(q13;q13). Virchows Archiv A Pathol. Anat. **415**: 377-382.

19. Turc-Carel, C., Limon, J., Dal Cin, P., Rao, U., Karakousis, C., and Sandberg, A.A. (1986). Cytogenetic studies of adipose tissue tumours. II. Recurrent reciprocal translocation t(12;16)(q13;p11) in myxoid liposarcomas. Cancer Genet. Cytogenet. **23**: 291-299.

20. Rodiguez, E., Sreekantaiah, C., Reuter, V.E., Motzer, R.J. and Chaganti, R.S.K. (1992). t(12;22)(q13;q13) and trisomy 8 are nonrandom aberrations in clear-cell sarcoma. Cancer Genet. Cytogenet. **64**: 107-110.

21. Reeves, B.R., Fletcher, C.D.M. and Gusterson, B.A. (1992). Translocation t(12;22)(q13;q13) is a nonrandom rearrangement in clear cell sarcoma. Cancer Genet. Cytogenet. **64**: 101-103.

22. Fletcher, J.A. (1992). Translocation (12;22)(q13-14;q12) is a nonrandom aberration in soft-tissue clear-cell sarcoma. Genes Chrom. Cancer **5**: 184.

23. Roberts, P., Browne, C.F., Lewis, I.J., Bailey, C.C., Spice, R.D., Williams, J. and Batcup, G. (1992). 12q13 Abnormality in rhabdomyosarcoma. A nonrandom Occurrence? Cancer Genet. Cytogenet. **60**: 135-140.

24. Schoenmakers, H.F.P.M., Mols, R., Wanschura, S., Kools, P.F.J., Geurts, J.M.W., Bartnitzke, S., Bullerdiek, J., Van den Berghe, H., and Van de Ven, W.J.M. (1994b). Identification, molecular cloning and characterization of the chromosome 12 breakpoint cluster region of uterine leiomyomas. Genes Chrom. & Cancer. **11**: 106-118.

25. Van de Ven, W.J.M., Schoenmakers, H.F.P.M., Wanschura, S., Kazmierczak, B., Kools, P.F.J., Geurts, J.M.W., Bartnitzke, S., Van den Berghe, H., and Bullerdiek, J. (1995). Molecular characterization of MAR, a multiple aberration region on human chromosome segment 12q13-q15 implicated in various solid tumors. Genes Chrom. & Cancer. **12**: 296-303. (Enclosed as "ANNEX 1")

26. Casalone, r. et al. (1991) Cytogenetic analysis reveals clonal proliferation of smooth muscle cells in etherosclerotic plaques. Hum. Gen. **87**, 139-143.

27. Vanni, R. et al. (1990) Atherosclerotic plaque as a benign tumor. Cancer Genet. Cytogenet. **47**, 273-274.

28. Giancotti, V. et al. Elevated levels of a specific class of nuclear phosphoproteins in cells transformed with ras and v-mos oncogenes and by co-transfection with c-myc and polyoma middle T genes. EMBO J. **6**, 1981-1987 (1987).

29. Warburton, D., Gersen, S., Yu, M-T., Jackson, C., Handelin, B. and Housman, D. (1990). Monochromosomal rodent-human hybrids from microcell fusion of human lymphoblastoid cells containing an inserted dominant selectable marker. Genomics **6**: 358-366.

30. Schoenmakers, H.F.P.M., Kools, P.F.J., Kazmierczak, B., Bullerdiek, J., Claussen, U., Horsthemke, B., Van den Berghe, H. and Van de Ven, W.J.M. (1993). Isolation of a somatic cell hybrid retaining the der(16)t(12;16)(q13;p11.2) from a myxoid liposarcoma cell line. Cytogenet. Cell Genet. **62**: 159-161.

31. Rychlik, W. and Rhoads, R.E. (1989). A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucleic Acids Res. **17**: 8543-8551.

32. Verbeek, J.S., Roebroek, A.J.M., Van den Ouweland, A.M.W., Bloemers, H.P.J. and Van de Ven, W.J.M. (1985). Human c-fms proto-oncogene: comparative analysis with an abnormal allele. Mol. Cell. Biol. **5**: 422-426.

33. Montgomery, K.T., LeBlanc, J.M., Tsai, P., McNinch, J.S., Ward, D.C., De Jong, P.J., Kucherlapati, R., and Krauter, K.S. (1993). Characterization of two chromosome 12 cosmid libraries and development STSs from cosmids mapped by FISH. Genomics **17**: 682-693

34. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

35. Dal Cin, P., Kools, P., Sciot, R., De Wever, I., Van Damme, B., Van de Ven, W. and Van den Berghe, H. (1993b). Molecular cytogenetic characterization of ring chromosomes in adipose tissue tumors. Cancer Genet. Cytogenet., **68**, 85-90.

36. Kievits, T., Dauwerse, J.G., Wiegant, J., Devilee, P., Breuning, M.H., Cornelisse, C.J. and van Ommen, G., Pearson, P.L. (1990). Rapid subchromosomal localization of cosmids by nonradioactive in situ hybridization. Cytogenet. Cell Genet. **53**: 134-136.

37. Albertsen, H.M., Abderrahim, H., Cann, H.M., Dausset, J., L Paslier, D., and Cohen, D. (1990). Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. Proc. Natl Acad. Sci. USA **87**: 4256-4260.

38. Chumakov, I., Rigault, P., Guillou, S., Ougen, P., Billaut, A., Guasconi, G., Gervy, P., LeGall, I., Soularue, P., Grinas, L., Bougueleret, L., Bellanné-Chantelot, C., Lacroix, B., Barillot, E., Gesnouin, P., Pook, S., Vaysseix, G., Frelat, G., Schmitz, A., Sambucy, J.L., Bosch, A., Estivill, X., Weissenbach, J., Vignal, A., Riethman, H., Cox, D., Patterson, D., Gardiner, K., Hattori, M., Sakaki, Y., Ichikawa, H., Ohki, M., L Paslier, D., Heilig, R., Aptonarakis, S., and Cohen, D. (1992). Continuum of overlapping clones spanning the entire human chromosome 21q. Nature **359**: 380-387.

39. Schoenmakers, H.F.P.M., Kools, P.F.J., Mols, R., Kazmierczak, B., Bartnitzke, S., Bullerdiek, J., Dal Cin, P., De Jong, P.J., Van den Berghe, H. and Van de Ven, W.J.M. (1994a). Physical mapping of chromosome 12q breakpoints in lipoma, pleomorphic salivary gland adenoma, uterine lei myoma, and myxoid liposarcoma. Genomics **20**: 210-222.

40. Chu, G., Vollrath, D. and Davis, R.W. (1986) Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234**: 1582-1585.

41. Geurts, J., Schoenmakers H.F.P.M., Mols, R., and Van de Ven, W.J.M. (1994). Improved procedure for rapid isolation and sequencing of DNA insert termini in yeast artificial chromosomes. Meth. Mol. Cell. Biol., **4**: 257-265.

42. Kools, P.F.J., Wanschura, S., Schoenmakers, E.F.P.M., Geurts, J.W.M., Mols, R., Kazmierczak, B., Bullerdiek, J., Van den Berghe, H. and Van de Ven, W.J.M. (1995). Identification of the chromosome 12 translocation breakpoint region of a pleomorphic salivary gland adenoma with t(1;12)(p22;q15) as the sole cytogenetic abnormality. Cancer Genet. Cytogenet., **79**: 1-7. (Enclosed as "ANNEX 2")

43. Kazmierczak, B., Wanschura, S., Rosigkeit, J., Meyer-Bolte, K., Uschinsky, K., Haupt, R., Schoenmakers, E.F.P.M., Bartnitzke, S., Van de Ven, W.J.M. and Bullerdiek, J. (1995). Molecular characterization of 12q14-15 rearrangements in three pulmonary chondroid hamartomas. Cancer Res., in press.

44. Kucherlapati, R., Craig, I., and Marynen, P. (1994). Report of the second international workshop on human chromosome 12 mapping 1994. Cytogenet. Cell Genet. **67**: 246-276

45. Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M. and Weissenbach, J. (1994). The 1993-94 Généthon human genetic linkage map. Nature Genetics **7**: 246-339.

46. Ulinowski, Z., Taylor, K., Griffin, D., Delhanty, J. and Wolfe, J. (1991). D12S56: a highly polymorphic locus on human chromosome 12q14. Ann. Hum. Genet. **55**: 279-282.

47. Trent, J.M., Olson, S. and Lawn, R.M. (1982) Chromosomal localization of human leukocyte, fibroblast, and immune interferon genes by means of in situ hybridization. Proc. Natl. Acad. Sci. U.S.A. **79**: 7809-7813.

48. Pizon, V., Lerosey, I., Chardin, P. and Tavitian, A. (1988). Nucleotide sequence of a human cDNA encoding a ras-related protein (rap1B). Nucleic Acids Res. **16**: 7719.

49. Adams, M.D., Dubnick, M., Kerlavage, A.R., Moreno, R., Kelley, J.M., Utterback, T.R., Nagle, J.W., Fields, C., and Venter, J.C. (1992). Sequence identification of 2,375 human brain genes. Nature **355**: 632-634.

50. Cohen, D., Chumakov, I., and Weissenbach, J. (1993). A first-generation physical map of the human genome. Nature **366**: 698-701.

51. Larsen, F., Gundersen, G., and Prydz, H. (1992a). Choice of enzymes for mapping based on CpG islands in the human genome. GATA **9**: 80-85.

52. Larsen, F., Gundersen, G., Lopez, R., and Prydz, H. (1992b). CpG islands as gene markers in the human genome. Genomics **13**: 1095-1107.

53. Lavia, P., MacLeod, D., and Bird, A. (1987). Coincident start sites for divergent transcripts at randomly selected CPG-rich island of mouse. EMBO J. **6**: 2773-2779.

54. Kazmierczak, B., Bartnitzke, S., Hartl, M. & Bullerdiek, J. In vitro transformation by the SV40 "early region" of cells from a human benign salivary gland tumor with a 12q13-q15 rearrangement. Cytogenet. Cell Genet. **53**, 37-39 (1990).

55. Albertsen, H.M., Adderrahim, H., Cann, H.M., Dausset, J., Le Paslier, D. & Cohen, D. Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. Proc. natl. Acad. Sci. U.S.A. **87**, 4256-4260 (1990).

56. Chumakov, I. et al. Continuum of overlapping clones spanning the entire human chromosome 21q. Nature **359**, 380-387 (1992).

57. Green, E.D. & Olson, M.V. Systematic screening of yeast artificial-chromosome libraries using the polymerase chain reaction. Proc. natl. Acad. Sci. U.S.A. **87**, 1213-1217 (1990).

58. Montgomery, K.T. et al. Characterization of two chromosome 12 cosmid libraries and development of STSs from cosmids mapped by FISH. Genomics **17**, 682-693 (1993).

59. Geurts, J.M.W., Schoenmakers E.F.P.M., Mols, R. & Van de Ven, W.J.M. Improved procedure for rapid isolation and sequencing of DNA insert termini in yeast artificial chromosomes. Meth. Mol. Cell. Biol. **4**, 257-265 (1994).

60. Nelson, D.L. et al. Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc. natl. Acad. Sci. U.S.A. **86**, 6686-6690 (1989).

61. Rychlik, W. & Rhoads, R.E. A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucl. Acids Res. **17**, 8543-8551 (1989).

62. Feinberg, A.P. & Vogelstein, B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**, 6-13 (1984).

63. Smith, M.W., Holmsen, A.L., Wei, Y.H., Peterson, M. & Evans, G.A. Genomic sequence sampling: a strategy for high resolution sequence-based physical mapping of complex genomes. Nature Genetics **7**, 40-47 (1994).

64. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. Basic local alignment search tool. J. Mol. Biol. **215**, 403-410 (1990).

65. Patel, U.A. et al. Expression and cDNA cloning of human HMGI-C phosphoprotein. Biochem. Biophys. Res. Commun. **201**, 63-70 (1994).

66. Bustin, M., Lehn, D.A. & Landsman, D. Structural features of the HMG chromosomal proteins and their genes. Biochim. Biophys. Acta **1049**, 231-243 (1990).

67. Manfioletti, G. et al. cDNA cloning of the HMGI-C phosphoprotein, a nuclear protein associated with neoplastic and undifferentiated phenotypes. Nucl. Acids Res. **19**, 6793-6797 (1991).

68. Wagner, M.J., Ge, Y., Siciliano, M. & Wells, D.E. A hybrid cell mapping panel for regional localization of probes to human chromsome 8. Genomics **10**, 114-125 (1991).

69. Friedman, M., Holth, L.T., Zoghbi, H.Y. & Reeves, R. Organization, inducible-expression and chromosome localization of the human HMG-I(Y) nonhistone protein gene. Nucl. Acids Res. **21**, 4259-4267 (1993).

70. Rabbitts, T.H. Chromosomal translocations in human cancer. Nature **372**, 143-149 (1994).

71. Yang-Yen, H.F. & Rothblum, L.I. Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis in vitro. Mol. Cell. Biol. **8**, 3406-3414 (1988).

72. Reeves, R., Langan, T.A. & Nissen, M.S. Phosphorylation of the DNA-binding domain of nonhistone high-mobility group I protein by cdc2 kinase: reduction of binding affinity. Proc. natl. Acad. Sci. U.S.A. **88**, 1671-1675 (1991).

73. Thanos, D. & Maniatis, T. The high mobility group protein HMGI(Y) is required for NF-■B-dependent virus induction of the human IFN-β gene. Cell **71**, 777-789 (1992).

74. Du, W. & Maniatis, T. The high mobility group protein HMGI(Y) can stimulate or inhibit DNA binding of distinct transcriptional factor ATF-2 isoforms. Proc. natl. Acad. Sci. U.S.A. **91**, 11318-11322 (1994).

75. Aizawa, S., Nishino, H., Saito, K., Kimura, K., Shirakawa, H. & Yoshida, M. Stimulation of transcription in cultured cells by high mobility group protein 1: Essential role of the acidic carboxy-terminal region. Biochemistry **33**, 14690-14695 (1994).

76. Mitelman F (1994): Catalog of Chromosome Aberrations in Cancer. 4th ed., New York, Wiley- Liss.

77. Miles, J.H., Zonanna, J., Mcfarlane, J., Aleck, K.A. & Bawle, E. Macrocephaly with hamartomas: Bannayan-Zonana syndrome. Am. J. Med. Genet. **19**, 225-234 (1984).

78. Crawford, A.W., Pino, J.D. & Beckerie, M.C.J. Cell. Biol., 124, 117-127 (1994)

79. Schmeichel, K.L. & Beckerie, M.C. Cell, 79, 211-219 (1994)

80. Sanchez-Garcia, I. & Rabbitts, T. (1994) The LIM domain: a new structural motif found in zinc-finger-like proteins. Trends in Genetics **10**(9), 315-320.

81. Compton, J. (1991). Nucleic acid sequence-based amplification. Nature **350**, 91-92.

ANNEX 1

GENES, CHROMOSOMES & CANCER 12:296–303 (1995)

Molecular Characterization of MAR, a Multiple Aberration Region on Human Chromosome Segment 12q13-q15 Implicated in Various Solid Tumors

Wim J.M. Van de Ven, Eric F.P.M. Schoenmakers, Sylke Wanschura, Bernd Kazmierczak, Patrick F.J. Kools, Jan M.W. Geurts, Sabine Bartnitzke, Herman Van den Berghe, and Jörn Bullerdiek

Center for Human Genetics, University of Leuven, Leuven, Belgium (W.J.M.V.d.V., E.F.P.M.S., P.F.J.K., J.M.W.G., H.V.d.B.); Center for Human Genetics, University of Bremen, Bremen, Germany (S.W., B.K., S.B., J.B.)

Chromosome arm 12q breakpoints in seven cell lines derived from primary pleomorphic salivary gland adenomas were mapped by FISH analysis relative to nine DNA probes. These probes all reside in a 2.8 Mb genomic DNA region of chromosome segment 12q13-q15 and correspond to previously published sequence-tagged sites (STS). Their relative positions were established on the basis of YAC cloning and long range physical and STS content mapping. The 12q breakpoints of five of the cell lines were found to be mapping within three different subregions of the 445 kb DNA interval that was recently defined as the uterine leiomyoma cluster region of chromosome 12 breakpoints (ULCR12) between STS RM33 and RM98. All seven breakpoints appeared to map within the 1.7 Mb DNA region between STS RM36 and RM103. Furthermore, the chromosome 12 breakpoints of three primary pleomorphic salivary gland adenomas were also found to be mapping between RM36 and RM103. Finally, FISH analysis of two lipoma cell lines with 12q13-q15 aberrations pinpointed the breakpoints of these to relatively small and adjacent DNA segments which, as well as those of two primary lipomas, appeared to be located also between RM36 and RM103. We conclude from the observed clustering of the 12q breakpoints of the three distinct solid tumor types that the 1.7 Mb DNA region of the long arm of chromosome 12 between RM36 and RM103 is a multiple aberration region which we designate MAR. *Genes Chromosom Cancer 12:296–303 (1995).* © 1995 Wiley-Liss, Inc.

INTRODUCTION

Chromosome translocations involving region q13-q15 of chromosome 12 have been observed in a wide variety of solid tumors (Mitelman, 1991). In subgroups of cytogenetically abnormal uterine leiomyomas (Nilbert and Heim, 1990; Pandis et al., 1991), pleomorphic salivary gland adenomas (Sandros et al., 1990; Bullerdiek et al., 1993), and benign adipose tissue tumors (Sreekantaiah et al., 1991), 12q13-q15 aberrations are frequently observed. In a recent study (Schoenmakers et al., 1994b), we identified and molecularly characterized ULCR12, the uterine leiomyoma cluster region of chromosome 12 breakpoints. In the present study, we focus on the chromosome arm 12q breakpoints in pleomorphic adenoma of the salivary glands, a benign epithelial tumor originating from the major or minor salivary glands. It is the most common type of salivary gland tumor and accounts for almost 50% of all neoplasms in these organs. About 85% of the tumors are found in the parotid gland, 10% in the minor salivary glands, and 5% in the submandibular gland (Seifert et al., 1986). Although many of these adenomas appear to have a normal karyotype, cytogenetic studies have also revealed recurrent specific chromosome anomalies (Sandros et al., 1990; Bullerdiek et al., 1993). Besides chromosome 8 aberrations, often translocations with a breakpoint in 8q12 with, as the most common aberration, a t(3;8)(p21;q12), aberrations of chromosome 12, usually translocations involving 12q13-q15, are also frequent. Non-recurrent clonal abnormalities have also been described. The frequent involvement of region 12q13-q15 in distinct solid tumor types suggests that this chromosomal region harbors gene(s) that might be implicated in the evolution of these tumors. Molecular cloning of the chromosome 12 breakpoints of these tumors and characterization of the junction fragments may therefore lead to the identification of such gene(s).

On the basis of fluorescence in situ hybridization (FISH) data, we previously reported that the chromosome 12 breakpoints in a number of cell lines derived from primary pleomorphic salivary gland adenomas (Kazmierczak et al., 1990; Schoenmakers et al., 1994a) are located on the long arm of chromosome 12 in the interval between loci D12S19 and D12S8 (Schoenmakers et al., 1994a). This DNA interval has been estimated to be about 7 cM (Keats et al., 1989; Craig et al., 1993). The interval con-

Received September 27, 1994; accepted November 7, 1994.

Address reprint requests to Dr. Wim J.M. Van de Ven, Center for Human Genetics, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium.

© 1995 Wiley-Liss, Inc.

taining the chromosome 12 breakpoints of these tumor cells was narrowed further by showing that all breakpoints mapped distally to the *CHOP* gene, which is directly affected by the characteristic t(12;16) translocation in myxoid liposarcomas (Aman et al., 1992; Crozat et al., 1993; Rabbitts et al., 1993) and is located between D12S19 and D12S8. In more recent studies (Kools et al., 1995), the chromosome 12 breakpoint of pleomorphic salivary gland adenoma cell line Ad-312/SV40 was pinpointed to a DNA region between sequence-tagged sites (STSs) RM110 and RM111, which is less than 165 kb in size. FISH evaluation of the chromosome 12 breakpoints of the other pleomorphic salivary gland adenoma cell lines indicated that they must be located proximally to the one in Ad-312/SV40, at a distance of more than 800 kb (Kools et al., 1995). These results pointed toward a possible dispersion of the chromosome 12 breakpoints over a relatively large genomic region on the long arm of chromosome 12.

Here, we report physical mapping of the chromosome 12 breakpoints in pleomorphic salivary gland adenoma cells from primary tumors as well as established tumor cell lines. The karyotypic anomalies observed in the cells were all different but always involved region q13-q15 of chromosome 12. Using DNA probes between D12S8 and *CHOP*, which corresponded to sequence-tagged sites (STSs) of a long-range physical map of a 6 Mb DNA region and were obtained during chromosome walking experiments, we performed FISH experiments and defined more precisely a major chromosome 12 breakpoint cluster region of pleomorphic salivary gland adenoma. This breakpoint cluster region appeared to overlap with ULCR12. Furthermore, we tested whether 12q13-q15 breakpoints of lipomas might also map within the same region as those of pleomorphic salivary gland adenoma and uterine leiomyoma.

TABLE 1. Chromosome 12 Aberrations in Primary Human Solid Tumors and Cell Lines*

	Aberration
Cell lines	
Ad-211/SV40	t(8;12)(q21;q13-q15)
Ad-248/SV40	ins(12;6)(q15;q16q21)
Ad-263/SV40	inv(12)(q15q24.1)
Ad-295/SV40	t(8;12;18)(p12;q14;p11.2)
Ad-302/SV40	t(7;12)(q31;q14)
Ad-366/SV40	inv(12)(p13q15)
Ad-386/SV40	t(12;14)(q13-q15;q13-q15)
Li-14/SV40	t(3;12)(q28;q13)
Li-538/SV40	t(3;12)(q27;q14)
LM-5.1/SV40	t(12;15)(q15;q24)
LM-30.1/SV40	t(12;14)(q15;q24)
LM-65/SV40	t(12;14)(q15;q24)
LM-67/SV40	t(12;14)(q13-q15;q24)
LM-100/SV40	t(12;14)(q15;q24)
LM-605/SV40	ins(12;11)(q14;q21qter)
LM-608/SV40	t(12;14)(q15;q24)
LM-609/SV40	t(12;14)(q15;q24)
Primary tumors	
Ad-386	t(12;14)(q15;q11.2)
Ad-396	t(3;12)
Ad-400	t(12;16)
Li-166	t(12;12)
Li-167	t(3;12)(q28;q14-q15)
LM-163.1	t(12;14)(q14;q24)
LM-163.2	t(12;14)(q14;q24)
LM-168.3	t(X;12)(q22;q15)
LM-192	t(2;3;12)(q35;p21;q14)
LM-196.4	t(12;14)(q14;q24)

*Ad, pleomorphic salivary gland adenoma; Li, lipoma; LM, uterine leiomyoma.

MATERIALS AND METHODS

Primary Solid Tumors and Derivative Cell Lines

Primary solid tumors including pleomorphic salivary gland adenomas, lipomas, and uterine leiomyomas were obtained from the University Clinics in Leuven, Belgium (Dr. I. De Wever); in Bremen, Germany (Dr. R. Chille); in Krefeld, Germany (Dr. J. Haubrich); and from the Institute of Pathology in Göteborg, Sweden (Dr. G. Stenman). For cell culturing and subsequent FISH analysis, tumor samples were finely minced, treated for 4–6 hours with 0.8% collagenase (Boehringer, Mannheim, FRG), and processed further for FISH analysis according to routine procedures.

Human tumor cell lines used in this study included the previously described pleomorphic salivary gland adenoma cell lines Ad-211/SV40, Ad-248/SV40, Ad-263/SV40, Ad-295/SV40, Ad-302/SV40, Ad-366/SV40, and Ad-386/SV40 (Kazmierczak et al., 1990; Schoenmakers et al., 1994a) and the lipoma cell lines Li-14/SV40 (Schoenmakers et al., 1994a) and the recently developed Li-538/SV40. Chromosome 12 aberrations found in these cell lines are listed in Table 1. Cells were propagated in TC199 culture medium with Eagle's salts supplemented with 20% fetal bovine serum.

DNA Probes

In the context of a human genome project focusing on the long arm of chromosome 12, we isolated cosmid clones cRM33, cRM36, cRM51, cRM69,

cRM72, cRM76, cRM98, cRM103, and cRM133, from chromosome 12-specific arrayed cosmid library LLNL12NC01 (Montgomery et al., 1993). Further details of these cosmid clones have been reported at the Second International Chromosome 12 Workshop (1994) and will be described elsewhere (Kucherlapati et al., 1994). Briefly, initial screenings were performed using a PCR-based screening strategy (Green and Olson, 1990), followed by filter hybridization analysis as the final screening step, as previously described (Schoenmakers et al., 1994b). The cosmid clones were isolated using STSs derived from YAC clones. STSs were obtained upon rescue of YAC insert-ends using a methodology involving vectorette-PCR followed by direct solid phase fluorescent sequencing of the PCR products (Geurts et al., 1994) or from inter-Alu PCR (Nelson et al., 1989). Cosmid clones were grown and handled according to standard procedures (Sambrook et al., 1989).

Cosmid clone cPK12qter, which maps to the telomeric region of the long arm of chromosome 12 (Kools et al., 1995), was used as a reference marker.

Chromosome Preparations and Fluorescence In Situ Hybridization

Metaphase cells of the pleomorphic salivary gland adenoma cell lines or normal human lymphocytes were prepared as described before (Schoenmakers et al., 1993). To unambiguously establish the identity of chromosomes in the FISH experiments, FISH analysis was performed after GTG-banding of the same metaphase spreads. GTG-banding was performed essentially as described by Smit et al. (1990). In situ hybridizations were carried out according to a protocol described by Kievits et al. (1990) with some minor modifications (Kools et al., 1994; Schoenmakers et al., 1994b). Cosmid and YAC DNA was labelled with biotin-11-dUTP (Boehringer Mannheim) or biotin-14-dATP (BRL, Gaithersburg) as described before (Schoenmakers et al., 1994b). Specimens were analyzed on a Zeiss Axiophot fluorescence microscope using a FITC filter (Zeiss). Results were recorded on Scotch (3M) 640 asa film.

RESULTS

FISH Mapping of 12q Breakpoints in Cell Lines of Pleomorphic Salivary Gland Adenoma

In previous studies (Schoenmakers et al., 1994a), we mapped the chromosome 12 breakpoints in a number of pleomorphic adenomas of the salivary glands relative to various DNA markers and established that these were all located proximal to locus D12S8 and distal to the *CHOP* gene. This region is somewhat smaller than the 7 cM region encompassed by linkage loci D12S8 and D12S19 (Keats et al., 1989). Using YAC cloning, a long range physical/STS map has been constructed covering most of that 7 cM region, as recently reported (Kucherlapati et al., 1994). Furthermore, numerous genomic clones (cosmid clones) have been isolated and their relative positions within this map established (Kucherlapati et al., 1994). Nine of these cosmids, including cRM33, cRM36, cRM51, cRM69, cRM72, cRM76, cRM98, cRM103, and cRM133, were used in FISH studies to establish the positions of the chromosome 12 breakpoints of the seven cell lines derived from pleomorphic adenomas of the salivary glands (Table 1). The relative mapping order of these nine cosmid clones, which cover a genomic region on the long arm of chromosome 12 of about 2.8 Mb, is indicated in Figure 1 and the results of FISH studies with the various cosmid probes are schematically summarized in the same figure. As an illustration, FISH results obtained with metaphase cells of cell line Ad-295/SV40 using cRM76 and cRM103 as probes are shown in Figure 2. It should be noted that for the identification of chromosomes, pre-FISH GTG-banding was used routinely. On the basis of such banding, hybridization signals could be assigned conclusively to chromosomes of known identity; this was of major importance for cases with cross- or background hybridization signals, as these were occasionally observed. When GTG-banding in combination with FISH analysis provided inconclusive results, either because of weak hybridization signals or rather vague banding, FISH experiments were performed with cosmid clone cPK12qter (Kools et al., 1995) as a reference probe.

FISH analysis of metaphase chromosomes of each of the seven pleomorphic salivary gland adenoma cell lines with cosmid cRM103 revealed that this cosmid mapped distal to the chromosome 12 breakpoints of all seven cell lines studied here. Metaphase chromosomes of six of the seven cell lines were also tested with probe cRM69 and, in two cases, with cRM51. The results of the latter experiments were always consistent with those obtained with cRM103. Similar FISH analysis with cRM36 as probe indicated that this probe mapped proximal to all the breakpoints. These results were always consistent with those obtained for five of the seven cell lines in experiments using cRM72. Altogether, the results of our FISH studies indi-

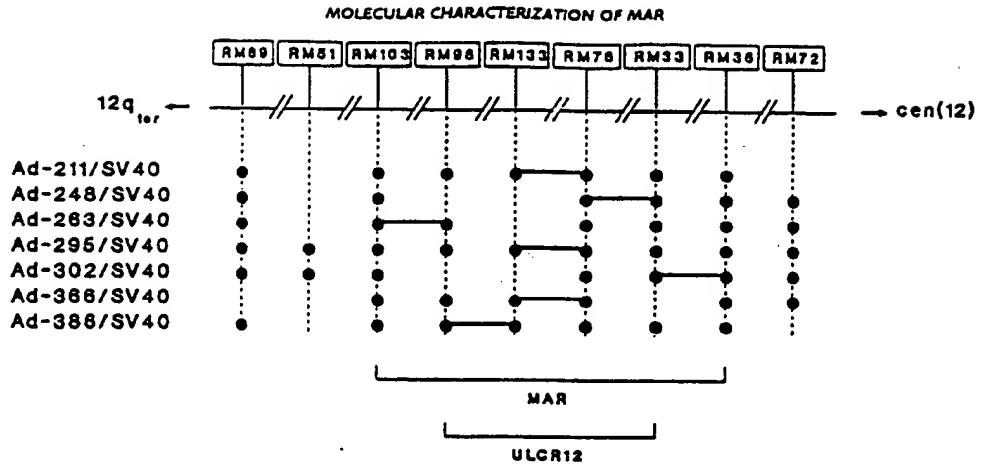


Figure 1. Schematic representation of FISH mapping data obtained for the seven pleomorphic salivary gland adenoma cell lines tested in this study. Cosmid clones which were used as probes in the FISH mapping studies map at sequence-tagged sites obtained from overlapping YAC clones. They are named after the acronyms of the STSs, as shown in the boxes, and the relative order of these is as presented. The DNA interval between RM69 and RM72 is estimated to be about 2.8 Mb. The solid lines indicate DNA intervals in which the breakpoints of the various cell lines are located. The dots indicate FISH experiments that were performed on metaphase chromosomes of the various cell lines using a cosmid clone corresponding to the STS indicated above these as molecular probe. The relative positions of MAR and ULCR12 are indicated in the lower part of the figure. Ad, pleomorphic salivary gland adenoma; MAR, multiple aberration region; ULCR12, uterine leiomyoma cluster region of chromosome 12 breakpoints.

cated that the chromosome 12 breakpoints of all seven cell lines map between cRM36 and cRM103, which spans a genomic region of about 1.7 Mb.

Fine Mapping of 12q Breakpoints in Cell Lines Derived From Pleomorphic Adenomas of the Salivary Glands

For subsequent fine mapping of the chromosome 12 breakpoints of the seven pleomorphic salivary gland adenoma cell lines, additional FISH studies were performed, as schematically summarized in Figure 1. The breakpoints of cell lines Ad-211/SV40, Ad-295/SV40, and Ad-366/SV40 appeared to be located in the DNA region between cRM76 and cRM133, which was estimated to be about 75 kb. The breakpoints of the four other cell lines were found in different areas of the 1.7 Mb region between cRM36 and cRM103. That of cell line Ad-248/SV40 in a DNA segment of about 270 kb between cRM33 and cRM76, that of Ad-263/SV40 in a DNA segment of about 1 Mb between cRM98 and cRM103, that of Ad-302/SV40 in a DNA segment of about 240 kb between cRM33 and cRM36, and that of Ad-386/SV40 in a DNA segment of about 100 kb between cRM98 and cRM133. In conclusion, these results indicated that the chromosome 12 breakpoints of most (5 out of 7) of the cell lines are dispersed over the 445 kb genomic region on the long arm of chromosome 12 between cRM33 and cRM98. It is important to note already here that precisely this region was recently shown to contain the chromosome 12q breakpoints in cell lines derived from primary uterine leiomyomas (see Fig. 3) and was therefore designated ULCR12 (Schoenmakers et al., 1994b). As this segment of the long arm of chromosome 12 is involved in at least two types of solid tumors (Schoenmakers et al., 1994b; this study) and, as we will show below, also in a third solid tumor type, we will from now on refer to the DNA interval between cRM36 and cRM103 as MAR (multiple aberration region).

FISH Mapping of 12q Breakpoints in Primary Pleomorphic Salivary Gland Adenomas

Our FISH studies on metaphase chromosomes of pleomorphic adenomas of the salivary glands presented so far were restricted to cell lines derived from primary tumors. Although it is reasonable to assume that the chromosome 12 breakpoints in cell lines are similar if not identical to the ones in the corresponding primary tumors, differences as a result of the establishment of cell lines or subsequent cell culturing cannot fully be excluded. Therefore, we have investigated whether the chromosome 12 breakpoints in three primary salivary gland adenomas were mapping to MAR as well. To test this possibility, a combination of cosmid clones

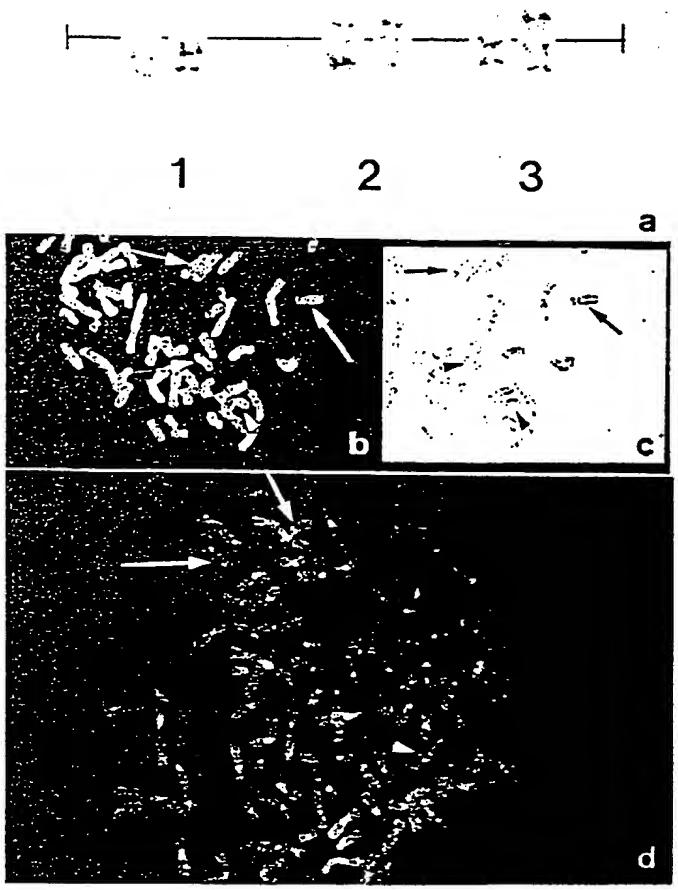


Figure 2. a: Partial karyotype of Ad-295/SV40 showing der(8), der(12), der(18), and the corresponding normal chromosomes. b: FISH analysis of metaphase chromosomes of Ad-295/SV40 cells using DNA of cosmid clone cRM76 as molecular probe. Hybridization signals on normal chromosome 12 (arrow) and der(12) (arrowhead). c: GTG-banding pattern of metaphase chromosomes of Ad-295/SV40 shown in b. d: FISH analysis of metaphase chromosomes of Ad-295/SV40 cells using DNA of cosmid clone cRM103 as molecular probe. Hybridization signals on normal chromosome 12 (arrow) and der(18) (arrowhead).

cRM33 and cRM103 was used as a molecular probe. In all three cases, this cosmid pool clearly spanned the chromosome 12 breakpoints (data not shown), indicating that these breakpoints were indeed localized within MAR. In a recent study (Wanschura et al., submitted for publication), it was reported that the chromosome 12 breakpoints of five primary uterine leiomyomas with 12q14-15 aberrations were all found to cluster within the 1.5 Mb DNA fragment (between cRM33 and cRM103), which is known to harbor the breakpoints of various cell lines derived from primary uterine leiomyomas (schematically summarized in Fig. 3). Consistent with the results of the breakpoint mapping studies using cell lines, the results with the two primary solid tumor types establish that the breakpoints of the primary tumor cells are also located in MAR.

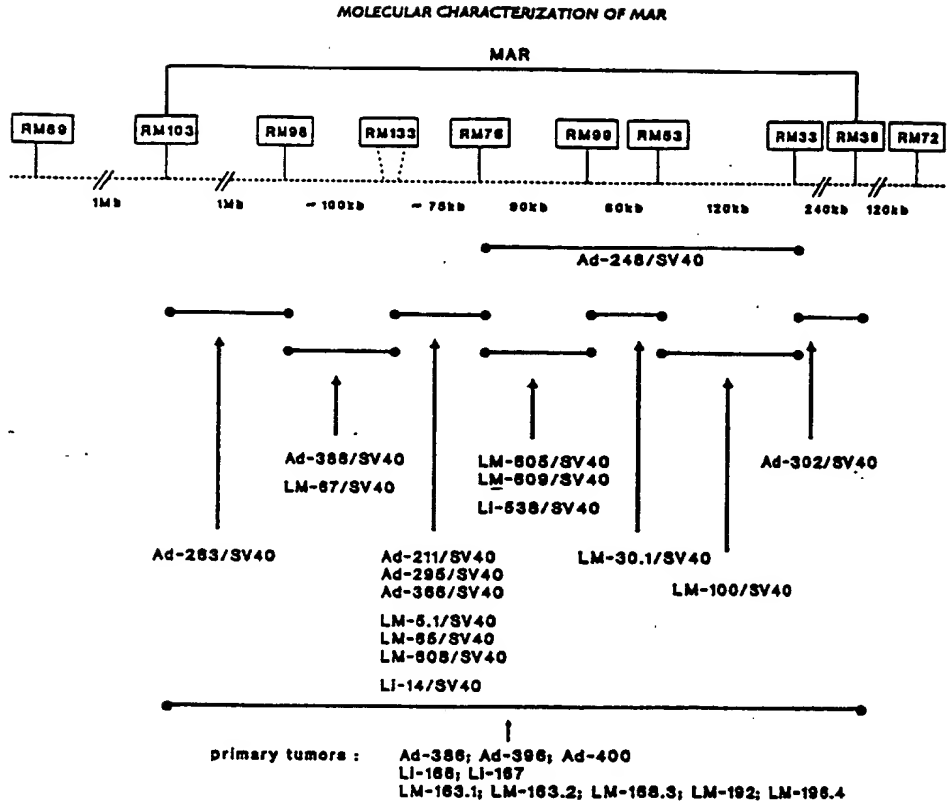


Figure 3. Schematic representation of chromosome 12 breakpoint mapping data obtained for primary pleomorphic salivary gland adenomas, uterine leiomyomas, and lipomas as well as cell lines derived from such solid tumors. Results are compared to data for primary uterine leiomyomas (Wanschura et al., submitted for publication) and cell lines derived from such tumors (Schoenmakers et al., 1994b). Cosmid clones which were used as probes in the FISH mapping studies correspond to sequence-tagged sites obtained from overlapping YAC clones. Cosmid clones were named after the acronyms of the STSs, as shown in the boxes, and the relative order of these is as presented. The estimated sizes of DNA intervals between STSs are indicated. Ad, pleomorphic salivary gland adenoma; LI, lipoma; LM, uterine leiomyoma.

Chromosome Segment 12q13-q15 Breakpoints of Lipomas Mapping Within MAR

To test the possibility that the chromosome 12 breakpoints of other solid tumors with 12q13-q15 aberrations also mapped within MAR, we studied two lipomas cell lines by FISH analysis—Li-14/SV40 and Li-538/SV40. The chromosome 12 aberrations of these two lipoma cell lines are given in Table 1. As molecular probes, cosmid clones cRM33, cRM53, cRM72, cRM76, cRM99, cRM103, and cRM133 were used. The breakpoint of Li-14/SV40 was mapped to the 75 kb DNA interval between RM76 and RM133, and that of Li-538/SV40 to the 90 kb interval between RM76 and RM99 (data not shown), as schematically illustrated in Figure 3. Similar FISH analysis of two primary lipomas using a mixture of cRM36 and cRM103 as molecular probe resulted in a hybridization pattern indicating that the mixture of probes detected sequences on either side of the breakpoints. These results are the first indications that also in lipoma, chromosome 12q13-q15 breakpoints occur that map within MAR. More lipoma cases should be tested to allow proper interpretation of this observation.

DISCUSSION

In this study, we have mapped the chromosome 12 breakpoints of three primary pleomorphic salivary gland adenomas as well as seven established cell lines derived from such tumors. All breakpoints appeared to be located in a previously molecularly cloned and characterized chromosome DNA segment on the long arm of chromosome 12, about 1.7 Mb in size, with five of them clustering

in a DNA interval of less than 500 kb. The 1.7 Mb DNA region apparently contains a major breakpoint cluster region for this type of tumor. In a previous study, we have described the characterization of the chromosome 12 breakpoint of pleomorphic salivary gland adenoma cell line Ad-312/SV40 (Kools et al., 1995). The breakpoint of this cell line is now known to map at a distance of more than 2 Mb distally to this major breakpoint cluster region reported here. It is possible that the Ad-312/SV40 breakpoint involves other pathogenetically relevant genetic sequences than those affected by the clustered breakpoints. However, the possibility should not yet be excluded that all the 12q13-q15 breakpoints in pleomorphic salivary gland adenomas mapped so far belong to the same category and are dispersed over a relatively large DNA region of this chromosome, reminiscent of the 11q13 breakpoints in B-cell malignancies (Raynaud et al., 1993). More precise pinpointing of the various breakpoints could shed more light on this matter.

Of importance is the observation that the DNA segment that harbors the clustered 12q breakpoints of pleomorphic salivary gland adenomas appears to coincide with the DNA region that was recently defined as the uterine leiomyoma cluster region of chromosome 12 breakpoints, known as ULCR12 (Schoenmakers et al., 1994b). Of further interest is the fact that this region of chromosome 12 also harbors breakpoints of primary lipomas and lipoma cell lines derived from primary tumors with 12q13-q15 aberrations. Altogether, the results of all these studies now clearly demonstrate that chromosome 12 breakpoints of three distinct solid tumor types map to the same 1.7 Mb genomic region on the long arm of chromosome 12, establishing this region to be a multiple aberration region. To reflect this characteristic, we have designated this DNA segment MAR.

Genetic aberrations involving chromosomal region 12q13-q15 have been implicated by many cytogenetic studies in a variety of solid tumors other than the three already mentioned. Involvement of 12q13-q15 has also been reported for endometrial polyps (Walter et al., 1989; Vanni et al., 1993), clear cell sarcomas characterized by recurrent t(12;22)(q13;q13) (Fletcher, 1992; Reeves et al., 1992; Rodriguez et al., 1992), a subgroup of rhabdomyosarcomas (Roberts et al., 1992) and hemangiopericytoma (Mandahl et al., 1993a), chondromatous tumors (Mandahl et al., 1989; Bridge et al., 1992; Hirabayashi et al., 1992; Mandahl et al., 1993b), and hamartoma of the lung (Dal Cin et al., 1993). Finally, several case reports of solid tumors with involvement of chromosome region 12q13-q15 have been published—e.g., tumors of the breast (Birdsal et al., 1992; Rohen et al., 1993), diffuse astrocytomas (Jenkins et al., 1989), and a giant-cell tumor of the bone (Noguera et al., 1989). On the basis of results of cytogenetic studies, no predictions could be made about the relative distribution of the breakpoints of these tumor types. In light of the results of the present study, it would be of interest to see whether the breakpoints of any of these solid tumors also map within or close to MAR. The various cosmid clones available now provide the means to test this readily.

The observation that 12q breakpoints of at least three different types of solid tumors map to the same DNA region is intriguing as it could be pointing towards the possibility that the same genetic sequences in MAR are pathogenetically relevant for tumor development in different tissues. If so, it is tempting to speculate that the gene(s) affected by the genetic aberrations might be involved in growth regulation. On the other hand, one cannot yet exclude the possibility that genetic sequences in MAR are not pathogenetically relevant, as the observed clustering of genetic aberrations in MAR could simply reflect genetic instability of this region, which becomes apparent in various solid tumors. To obtain more insight into this matter, the genes residing in MAR should be identified and characterized, and this can be achieved by various approaches using several techniques (Parrish and Nelson, 1993).

ACKNOWLEDGMENTS

The constructive support of managing director G. Everaerts is greatly acknowledged. The authors would like to thank P. Dal Cin, J. Haubrich, R. Hille, G. Stenman, and I. De Wever for providing the solid tumor specimens studied in the present report; C. Huysmans, E. Meyen, K. Meyer-Bolte, R. Mols, and M. Willems for excellent technical assistance; and M. Leys for artwork. This work was supported in part by the EC through the Biomed 1 program "Molecular Cytogenetics of Solid Tumours," the "Geconcerteerde Onderzoekacties 1992–1996," the National Fund for Scientific Research (NFWO; Kom op tegen Kanker), the "ASLK-programma voor Kankeronderzoek," the "Schwerpunktprogramm: Molekulare und Klassische Tumorcytogenetik" of the Deutsche Forschungsgemeinschaft, and the Tönjes-Vagt Stiftung. This text presents results of the Belgian programme on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors. J.M.W. Geurts is an "Aspirant" of the National Fund for Scientific Research (NFWO; Kom op tegen Kanker).

REFERENCES

Aman P, Ron D, Mandahl N, Fioretos T, Heim S, Arheden K, Willen H, Rydholm A, Mitelman F (1992) Rearrangement of the transcription factor gene *CHOP* in myxoid liposarcomas with t(12;16)(q13;p11). Genes Chromosom Cancer 5:278–285.

Birdsal SH, MacLennan KA, Gusterson BA (1992) t(6;12)(q23;q13) and t(10;16)(q22;p11) in a phyllodes tumor of the breast. Cancer Genet Cytogenet 60:74–77.

Bridge JA, Persons DL, Neff JR, Bhatia P (1992) Clonal karyotypic aberrations in enchondroma. Cancer Detect Prev 16:215–219.

Bullerdiek J, Wobst G, Meyer-Bolte K, Chilla R, Haubrich J, Thode B, Bartnitzke S (1993) Cytogenetic subtyping of 220 salivary gland pleomorphic adenomas: correlation to occurrence, histological subtype, and in vitro cellular behavior. Cancer Genet Cytogenet 65:27–31.

Craig IW, Gedde-Dahl T, Gemmill R, Kucherlapati R (1993) Report of the committee on the genetic constitution of chromosome 12. Genome Prior Rep 1:402–418.

Crozat A, Aman P, Mandahl N, Ron D (1993) Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640–644.

Dal Cin P, Kools P, De Jonge I, Moerman Ph, Van de Ven W, Van den Berghe H (1993) Rearrangement of 12q14–15 in pulmonary chondroid hamartoma. Genes Chromosom Cancer 8:131–133.

Fletcher JA (1992) Translocation (12;22)(q13–14;q12) is a nonrandom aberration in soft-tissue clear-cell sarcoma. Genes Chromosom Cancer 5:184.

Geurts JMW, Schoenmakers HFPM, Mols R, Van de Ven WJM (1994) An improved procedure to quickly isolate and sequence the termini of DNA inserts of yeast artificial chromosomes. Methods Mol Cell Biol 4:257–265.

Green ED, Olson MV (1990) Systematic screening of yeast artificial-chromosome libraries using the polymerase chain reaction. Proc Nad Acad Sci USA 87:1213–1217.

Hirabayashi Y, Yoshida MA, Ikeuchi T, Ishida T, Kojima T, Higaki S, Machinami R, Tonomura A (1992) Chromosome rearrangements at 12q13 in two cases of chondrosarcomas. Cancer Genet Cytogenet 60:35–40.

Jenkins RB, Kimmell DW, Moertel CA, Schulz CA, Menezes RM, Scheithuer B, Kelly PJ, Dewald GW (1989) Recurrent cytogenetic abnormalities in 80 gliomas. Cytogenet Cell Genet 51:1019.

Kazmierczak B, Bartnitzke S, Hartl M, Bullerdiek J (1990) In vitro transformation by the SV40 "early region" of cells from a human benign salivary gland tumour with a 12q13→q15 rearrangement. Cytogenet Cell Genet 53:37–39.

Keats B, Ott J, Connelly M (1989) Report of the committee on linkage and gene order. Cytogenet Cell Genet 51:459–502.

Kievits T, Dauwerse JG, Wiegant J, Devilee P, Breuning MH, Cornelisse CJ, van Ommen G, Pearson PL (1990) Rapid subchromosomal localization of cosmids by nonradioactive in situ hybridization. Cytogenet Cell Genet 53:134–136.

Kools PFJ, Roebroek AJM, Van de Velde HJK, Marynen P, Bullerdiek J, Van de Ven WJM (1993) Regional mapping of the human *NSP* gene to chromosome 14q21–q22 by fluorescence in situ hybridization. Cytogenet Cell Genet 66:48–50.

Kools PFJ, Wanschura S, Schoenmakers EFPM, Geurts JWM, Mols R, Kazmierczak B, Bullerdiek J, Van den Berghe H, Van de Ven WJM (1995) Identification of the chromosome 12 translocation breakpoint region of a pleomorphic salivary gland adenoma with t(1;12)(p22;q15) as the sole cytogenetic abnormality. Cancer Genet Cytogenet (in press).

Kucherlapati R, Craig I, Marynen P (1994) Report of the second international workshop on human chromosome 12 mapping 1994. Cytogenet Cell Genet 67:245–276.

Mandahl N, Heim S, Arheden K, Rydholm A, Willen H, Mitelman F (1989) Chromosomal rearrangements in chondromatous tumors. Cancer 65:242–248.

Mandahl N, Orndal C, Heim S, Willen H, Rydholm A, Bauer HCF, Mitelman F (1993a) Aberrations of chromosome segment 12q13–15 characterize a subgroup of hemangiopericytomas. Cancer 71:3009–3013.

Mandahl N, Willen H, Rydholm A, Mitelman F (1993b) Rearrangement of band q13 on both chromosomes 12 in a periosteal chondroma. Genes Chromosom Cancer 6:121–123.

Mitelman F (1991) Catalog of Chromosome Aberrations in Cancer, 4th ed. New York: Alan R. Liss.

Montgomery KT, LeBlanc JM, Tsai P, McNinch JS, Ward DC, De Jong PJ, Kucherlapati R, Krauter KS (1993) Characterization of two chromosome 12 cosmid libraries and development of STSs from cosmids mapped by FISH. Genomics 17:682–693.

Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT (1989) Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc Nad Acad Sci USA 86:6686–6690.

Nilbert M, Heim S (1990) Uterine leiomyoma cytogenetics. Genes Chromosom Cancer 2:3–13.

Noguera R, Llombart-Bosch A, Lopez-Gines C, Cardá C, Fernandez CI (1989) Giant-cell tumor of bone, stage II, displaying translocation t(12;19)(q13;q13). Virchows Archiv [A] 415:377–382.

Pandis N, Heim S, Bardi G, Flodérus U-M, Willén H, Mandahl N, Mitelman F (1991) Chromosome analysis of 96 uterine leiomyomas. Cancer Genet Cytogenet 55:11–18.

Parrish JE, Nelson DL (1993) Methods for finding genes a major rate-limiting step in positional cloning. GAT 10:29–41.

Rabbitts TH, Forster A, Larson R, Nathan P (1993) Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcoma. Nature Genet 4:175–180.

Raynaud SD, Bekri S, Leroux D, Grosgeorge J, Klein B, Bastard C, Gaudray P, Simon MP (1993) Expanded range of 11q13 breakpoints with differing patterns of cyclin D1 expression in B-cell malignancies. Genes Chromosom Cancer 8:80–87.

Reeves BR, Fletcher CDM, Gusterson BA (1992) Translocation t(12;22)(q13;q13) is a nonrandom rearrangement in clear cell sarcoma. Cancer Genet Cytogenet 64:101–103.

Roberts P, Browne CF, Lewis IJ, Bailey CC, Spice RD, Williams J, Barrup G (1992) 12q13 Abnormality in rhabdomyosarcoma. A nonrandom occurrence? Cancer Genet Cytogenet 60:135–140.

Rodriguez E, Sreekantaiah C, Reuter VE, Mercer RJ, Chaganti RSK (1992) t(12;22)(q13;q13) and trisomy 8 are nonrandom aberrations in clear-cell sarcoma. Cancer Genet Cytogenet 64:107–110.

Rohen C, Bonk U, Staats B, Bartnitzke S, Bullerdiek J (1993) Two human breast tumors with translocations involving 12q13–15 as the sole cytogenetic abnormality. Cancer Genet Cytogenet 69:68–71.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press.

Sandros J, Stenman G, Mark J (1990) Cytogenetic and molecular observations in human and experimental salivary gland tumours. Cancer Genet Cytogenet 44:153–167.

Schoenmakers HFPM, Kools PFJ, Kazmierczak B, Bullerdiek J, Claussen U, Horsthemke B, Van den Berghe H, Van de Ven WJM (1993) Isolation of a somatic cell hybrid retaining the der(16) t(12;16)(q13;p11.2) from a myxoid liposarcoma cell line. Cancer Genet Cytogenet 62:159–161.

Schoenmakers HFPM, Kools PFJ, Mols R, Kazmierczak B, Bartnitzke S, Bullerdiek J, Dal Cin P, De Jong PJ, Van den Berghe H, Van de Ven WJM (1994a) Physical mapping of chromosome 12q breakpoints in lipoma, pleomorphic salivary gland adenoma, uterine leiomyoma, and myxoid liposarcoma. Genomics 20:210–222.

Schoenmakers HFPM, Mols R, Wanschura S, Kools PFJ, Geurts JMW, Bartnitzke S, Bullerdiek J, Van den Berghe H, Van de Ven WJM (1994b) Identification, molecular cloning and characterization of the chromosome 12 breakpoint cluster region of uterine leiomyomas. Genes Chromosom Cancer 11:106–118.

Second International Chromosome 12 Workshop, New Haven, CT, USA, June 20–22, 1994.

Seifert G, Miehlke A, Haubrich J, Chilla R (1986) Diseases of the Salivary Glands. Pathology, Diagnosis, Treatment. Facial Nerve Surgery. Translated by P.M. Stell. Stuttgart, New York: Thieme, pp 182–194.

Smit VTHBM, Wessels JW, Mollevanger P, Schrier PI, Raap AK, Beverstock GC, Cornelisse CJ (1990) Combined GTG-banding and nonradioactive in situ hybridization improves characterization of complex karyotypes. Cytogenet Cell Genet 54:20–23.

Sreekantaiah C, Leong SLP, Karakousis CP, McGee DL, Rapoport WD, Villar HV, Neal D, Fleming S, Waakel A, Herrington PN, Carmona R, Sandberg AA (1991) Cytogenetic profile of 109 lipomas. Cancer Res 51:422–433.

Vanni R, Dal Cin P, Marras S, Moerman Ph, Andria M, Valdes E, Depresz J, and Van den Berghe H (1993) Endometrial polyp: Another benign tumor characterized by 12q13–15 changes. Cancer Genet Cytogenet 68:32–33.

Walter TA, Xuan Fan S, Medchill MT, Berger CS, Decker H-JH, Sandberg AA (1989) Inv(12)(p11.2q13) in an endometrial polyp. Cancer Genet Cytogenet 41:99–103.

Wanschura S, Belge G, Stenman G, Kools P, Dal Cin P, Schoenmakers E, Huysmans C, Bartnitzke S, Van de Ven W, and Bullerdiek J (submitted for publication) Mapping of the translocation breakpoints of primary pleomorphic adenomas and lipomas within a common region of chromosome 12.

ANNEX 2

LEAD ARTICLE

Identification of the Chromosome 12 Translocation Breakpoint Region of a Pleomorphic Salivary Gland Adenoma with t(1;12)(p22;q15) as the Sole Cytogenetic Abnormality

Patrick F. J. Kools, Sylke Wanschura, Eric F. P. M. Schoenmakers, Jan M. W. Geurts, Raf Mols, Bernd Kazmierczak, Jörn Bullerdiek, Herman Van Den Berghe, and Wim J. M. Van de Ven

ABSTRACT: *Cell line Ad-312/SV40, which was derived from a primary pleomorphic salivary gland adenoma with t(1;12)(p22;q15), was used in fluorescence in situ hybridization (FISH) analysis to characterize its translocation breakpoint region on chromosome 12. Results of previous studies have indicated that the chromosome 12 breakpoint in Ad-312/SV40 is located proximally to locus D12S8 and distally to the CHOP gene. We here describe two partially overlapping yeast artificial chromosome (YAC) clones, Y4854 (500 kbp) and Y9091 (460 kbp), which we isolated in the context of a chromosome walking project with D12S8 and CHOP as starting points. We present a composite long-range restriction map encompassing the inserts of these two YAC clones and show by FISH analysis that both YACs span the chromosome 12 breakpoint as present in Ad-312/SV40 cells. Subsequently, we have isolated cosmid clones corresponding to various sequence-tagged sites (STSs) mapping within the inserts of these YAC clones. These included cRM51, cRM69, cRM85, cRM90, cRM91, cRM110, and cRM111. In FISH studies, cosmid clones cRM85, cRM90, and cRM111 appeared to map distally to the chromosome 12 breakpoint, whereas cosmid clones cRM51, cRM69, cRM91, and cRM110 were found to map proximally to it. These results assign the chromosome 12 breakpoint in Ad-312/SV40 to a DNA region of less than 185 kbp. FISH evaluation of the chromosome 12 breakpoints in five other pleomorphic salivary gland adenoma cell lines indicated that these are located proximally to the one in Ad-312/SV40, at a distance of more than 0.9 Mbp from STS RM91. These results, while pinpointing a potentially critical region on chromosome 12, also provide evidence for the possible involvement of 12q13–q15 sequences located elsewhere.*

INTRODUCTION

.eomorphic salivary gland adenoma constitutes a benign epithelial tumor that originates from the major and minor salivary glands. It is the most common type of salivary gland tumor and accounts for almost 50% of all neoplasms in these organs; 85% of the tumors are found in the parotid gland, 10% in the minor salivary glands, and 5% in the submandibular gland [1]. About 50% of these adenomas appear to have a normal karyotype but cytogenetic studies have also revealed recurrent specific chromosome anomalies [2, 3]. Frequently observed anomalies include aberrations of chromosome 8, usually involving the 8q12–q13 region, with the most common aberration being a t(3;8)(p21;q12), and aberrations of chromosome 12, usually translocations involving region 12q13–q15. Non-recurrent clonal chromosome abnormalities have also been reported. The highly specific pattern of chromosome rearrangements with consistent breakpoints at 8q12–q13 and 12q13–q15 suggests that these chromosomal regions harbor genes that might be implicated in the development of these tumors. Molecular cloning of the chromosome breakpoints and characterization of their junction fragments may lead to the identification of pathogenetically relevant genes. At present, no such molecular data have yet been reported for these tumors.

On the basis of fluorescence in situ hybridization (FISH) data, the chromosome 12 breakpoints in six pleomorphic salivary gland adenoma cell lines were recently shown to be mapping to region 12q13–q15, more precisely, to the genomic interval between loci D12S19 and D12S8 [4, 5]. The sex-

From the Center for Human Genetics (P. F. J. K., E. F. P. M. S., J. M. W. G., R. M., H. V. D. B., W. J. M. V. D. V.), University of Leuven, Leuven, Belgium, and the Center for Human Genetics (S. W., B. K., J. B.), University of Bremen, Bremen, Germany.

P. F. J. Kools and Sylke Wanschura contributed equally to this study and must be considered joint first authors.

Address reprint requests to: Dr. Wim J. M. Van de Ven, Center for Human Genetics, University of Leuven, Herestraat 49, B-3000, Leuven, Belgium.

Received April 13, 1994; accepted July 6, 1994.

Cancer Genet Cytogenet 79:1–7 (1995)
© Elsevier Science Inc., 1995
655 Avenue of the Americas, New York, NY 10010

0165-4608/95/$9.50
SSDI 0165-4608(94)00137-Z

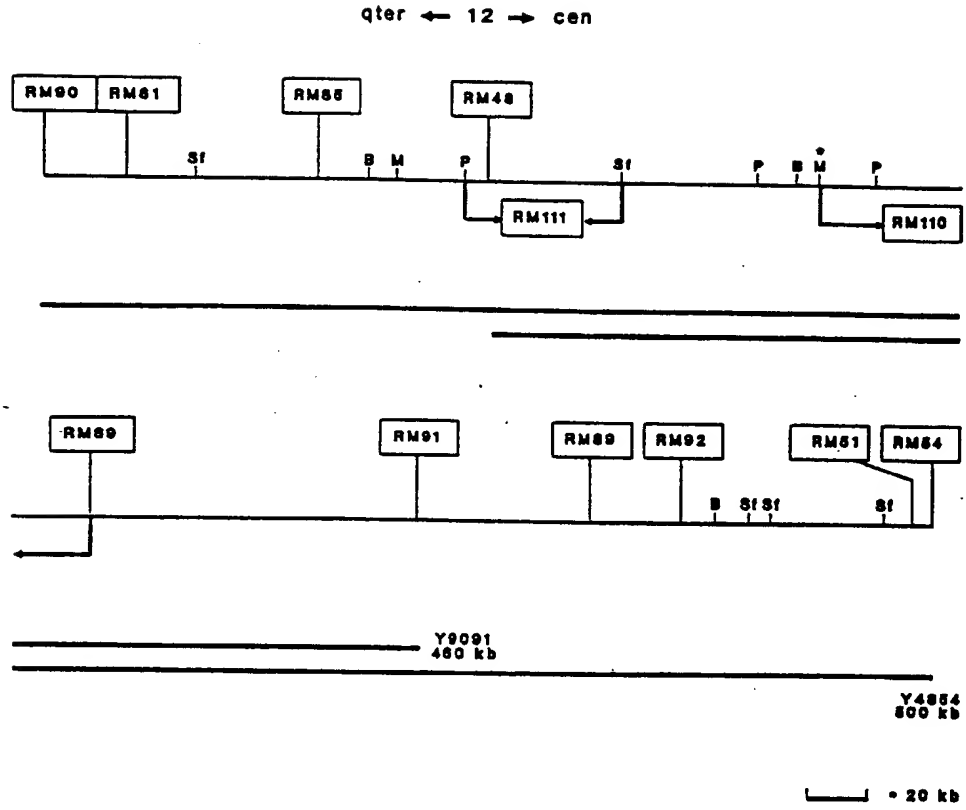


Figure 1 Composite physical map of the overlapping DNA inserts of YAC clones Y4854 and Y9091. Sizes of the DNA inserts are indicated. The relative positions of the YAC clones are represented by bars below the long-range physical map. Sequence-tagged sites (STSs) corresponding to end-clones of YACs, including YACs not shown here, are indicated by boxed RM codes above the restriction map. STSs obtained from inter-Alu-PCR products are given below the restriction map and the DNA regions to which they have been mapped are marked by arrows. B: *BssHII*; M: *MluI*; P: *PvuI*; Sf: *SfiI*. A polymorphic *MluI* site is marked by an asterisk.

averaged genetic size of this genomic DNA interval was reported at HGM10 to be 7 cM [6]. We also reported that the chromosome 12 breakpoints in salivary gland adenomas map distally to the *CHOP* gene [5], which supports an earlier study indicating that the 12q13–q15 translocation breakpoints in pleomorphic salivary gland adenomas are different from that in myxoid liposarcoma [7]. Here, we report the physical mapping of the chromosome 12 breakpoint in pleomorphic salivary gland adenoma cell line Ad-312/SV40, which carries a t(1;12)(p22;q15) as the only cytogenetic abnormality.

MATERIALS AND METHODS

Tumor Cell Lines

Human tumor cell lines used in this study included the previously described pleomorphic salivary gland adenoma cell lines Ad-248/SV40, Ad-263/SV40, Ad-293/SV40, Ad-302/SV40, Ad-312/SV40, and Ad-368/SV40 [5, 8]. Cells were cultivated in TC199 culture medium with Earle's salts supplemented with 20% fetal bovine serum. Other cell lines used in this study included somatic cell hybrid PKB9-12, which contains chromosome 12 as the sole human chromosome in a hamster genetic background [9], and somatic cell hybrid LIS-3/SV40/A9-B4 [4]. The latter cell line was obtained upon fusion of the myxoid liposarcoma cell line LIS-3/SV40, which carries the specific t(12;16)(q13;p11.2), with mouse A9 cells. This somatic cell hybrid was previously shown to contain der(16) but neither der(12) nor the normal chromosome 12 [4]. PKB9-12 and LIS-3/SV40/A9-B4 cells were grown in DME-F12 medium supplemented with 10% fetal bovine serum. Cell lines were analyzed by standard cytogenetic techniques at regular intervals.

Isolation of YAC and Cosmid Clones

In the context of human genome mapping studies, which will be described in detail elsewhere (Schoenmakers et al., in preparation), we isolated YAC clones Y4854 and Y9091 from the first-generation CEPH YAC library [10], and cosmid clones cRM51, cRM69, cRM85, cRM90, cRM91, cRM103, cRM110, and cRM111 from the chromosome-12-specific arrayed cosmid library LLNLNC01 [11]. YAC and cosmid clones were isolated as described before [5]. Initial screenings of the YAC, as well as the cosmid library, were performed using a screening strategy involving the polymerase chain reaction (PCR) [12]. Filter hybridization analysis was used as the final screening step, as previously described [5]. Cosmid clones were isolated using STSs and those corresponding to STSs within the inserts of YAC clones Y4854 and Y9091 are indicated in Figure 1. STSs were obtained via rescue of YAC insert end-sequences using a vectorette-PCR procedure [13] or Alu-PCR [14, 15]. PCR products were sequenced directly via solid-phase fluorescent sequencing. Cosmid clones were grown and handled according to standard procedures [16]. YAC clones were characterized by pulsed-field gel electrophoresis [17], restriction mapping, and hybridization, as previously described [5].

Chromosome Preparations and Fluorescence in Situ Hybridization

Cells from the pleomorphic salivary gland adenoma tumor cell lines were treated with Colcemid (0.04 μg/ml) for 30 min and then harvested according to routine methods. Metaphase spreads of the tumor cells were prepared as described before [4]. To establish the identity of chromosomes in the FISH experiments, FISH analysis was performed after G-banding of the same metaphase spreads. G-banding was performed essentially as described by Smit et al. [18]. In situ hybridizations were carried out according to a protocol described by Kievits et al. [19] with some minor modifications [5, 20]. Cosmid and YAC DNA was labeled with biotin-11-dUTP (Boehringer Mannheim) or biotin-14-dATP (BRL, Gaithersburg), as described earlier [5]. Chromosomes were counterstained with propidium iodide and analyzed on a Zeiss Axiophot fluorescence microscope using a FITC filter (Zeiss). Results were recorded on Scotch (3M) 640ASA film.

RESULTS

Isolation and Characterization of YAC Clones Spanning the Chromosome 12 Breakpoint of Pleomorphic Salivary Gland Adenoma Cell Line Ad-312/SV40

In previous studies [5], we mapped the chromosome 12 breakpoints of six pleomorphic salivary gland adenoma cell lines proximally to locus D12S8 and distally to *CHOP*. The DNA interval between these loci is somewhat smaller than 7 cM (estimated distance between the loci D12S8 and D12S19 [6]) but still substantially large. To molecularly define the translocation breakpoint of Ad-312/SV40, we have performed human genome mapping studies on the DNA interval between locus D12S8 and the *CHOP* gene. In the process of directional chromosome walking starting from D12S8 and the *CHOP* gene, we obtained overlapping YAC clones Y9091 and Y4854. The DNA insert of Y9091 appeared to be 460 kbp and that of Y4854, 500 kbp. Moreover, as we will demonstrate below, the DNA insert of each YAC clone appeared to span the chromosome 12 breakpoint of Ad-312/SV40. A long-range restriction map of the inserts of these YAC clones was made using pulsed-field gel electrophoresis and hybridization analysis (Fig. 1). On the basis of STS content mapping and Southern blot analysis, the inserts of YAC clones Y9091 and Y4854 appeared to overlap, as indicated in Figure 1. The tested STSs correspond to end-sequences of other overlapping YAC clones not shown here or to sequences obtained via inter-Alu-PCR. Of these, RM90 and RM91 represent such end-clone STSs of YAC Y9091, and RM48 and RM54 of Y4854, whereas RM110 and RM111 represent STSs derived from inter-Alu-PCR. For a number of STSs mapping within the inserts of YAC clones Y4854 and Y9091, corresponding cosmid clones were isolated for use in FISH analysis, e.g., cRM51, cRM69, cRM85, cRM90, cRM91, cRM110, and cRM111.

The inserts of the two overlapping YAC clones are most likely not chimeric, as was deduced from the following observations. FISH analysis of metaphase chromosomes of normal human lymphocytes with Y4854 or Y9091 DNA as molecular probe revealed hybridization signals only in chromosome region 12q13–q15. For Y9091, this was confirmed further by observations made in FISH studies in which cosmid clone cRM90 or cRM91 was used as probe; the DNA insert of each of these two cosmids corresponds to the alternative end-sequences of YAC clone Y9091. Finally, the end-sequence STSs of Y9091 appeared to map to chromosome 12 and distally to the *CHOP* gene, as was established by PCR analysis on PK89-12 DNA, which contains human chromosome 12 as the sole human chromosome in a hamster genetic background, and LIS-3/SV40/A9-B4 DNA, which was previously shown to contain der(18), from the specific t(12;18) of myxoid liposarcoma, but neither der(12) nor the normal chromosome 12 [4]. From the chromosome walking studies, we concluded that the overlapping inserts of the two YAC clones represent a DNA region of about 640 kbp, which is located on chromosome 12q between D12S8 and *CHOP*. As the 640-kbp composite long-range restriction map of the YAC contig was constructed with at least double coverage of the entire region, it is not unreasonable to assume that the 640-kbp region is contiguous with the chromosomal DNA, although microdeletions cannot be excluded at this point.

Chromosome walking was routinely evaluated by FISH mapping of YAC clones or cosmid clones corresponding to YAC insert sequences. It should be noted that for the identification of chromosomes, G-banding was used in most cases. On the basis of such G-banding, hybridization signals could be assigned conclusively to chromosomes of known identity; this was also of importance for the cases with cross- or background hybridization signals that were occasionally observed. G-banding prior to FISH analysis sometimes resulted in rather weak hybridization signals or rather vague banding patterns. Therefore, we performed FISH experiments in which the YAC and cosmid clones to be evaluated were used in combination with a reference probe. Cosmid clone cPK12qter, which was serendipitously obtained during screening of a cosmid library, was selected as reference marker. FISH analysis of metaphase chromosomes of nor-

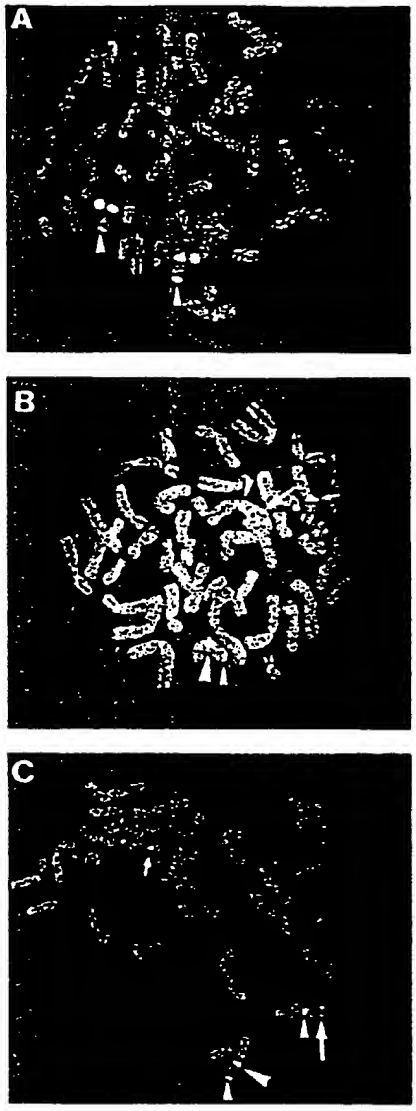


Figure 2 A) Mapping of cosmid clone cPK12qter to the telomeric region of the long arm of chromosome 12. Centromere 12-specific probe pα12H8 was used to establish the identity of chromosome 12. FISH analysis was performed on metaphase chromosomes of control human lymphocytes. Hybridization signals of cPK12qter are marked with small arrowheads, those of the centromere 12-specific probe with asterisks. B. C) FISH analysis of metaphase chromosomes of Ad-312/SV40 cells using DNA of YAC clone Y4854 (B) or Y9091 (C) as molecular probe in combination with cosmid clone cPK12qter as reference marker. Hybridization signals of the YAC clones on chromosome 12 are indicated by large arrowheads; those on der(1) by large arrows, and those on der(12) by small arrows, respectively. The hybridization signals of cosmid clone cPK12qter are indicated by small arrowheads.

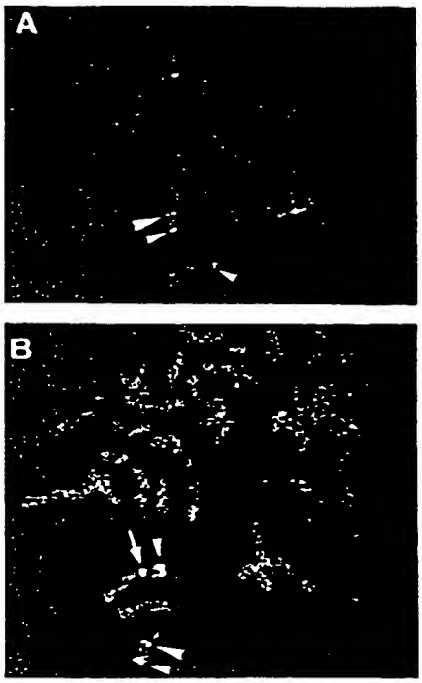


Figure 3 FISH analysis of metaphase chromosomes of Ad-312/SV40 cells using DNA of cosmid clone cRM69 (A), or cRM111 (B) as molecular probe in combination with cosmid clone cPK12qter as reference marker. The position of the hybridization signals of cPK12qter are indicated by small arrowheads. In (A), the position of the hybridization signal of cRM69 on normal chromosome 12 is indicated by a large arrowhead, and that on der(12) with a small arrow. In (B), the position of the hybridization signal of cRM111 on normal chromosome 12 is indicated by a large arrowhead, and that on der(1) with a large arrow.

mal lymphocytes (Fig. 2A) revealed that cPK12qter maps to the telomeric region of the long arm of chromosome 12. To identify chromosome 12 in this experiment, centromere 12-specific probe pα12H8 [21] was used. FISH analysis of metaphase chromosomes of Ad-312/SV40 cells using YAC clone Y4854 (Fig. 2B) or Y9091 (Fig. 2C) in combination with reference probe cPK12qter revealed, in both cases, hybridization signals of the YAC insert on der(1) as well as der(12). We concluded from these results that the insert DNA of each

Chromosome 12 Breakpoint of a Salivary Gland Adenoma

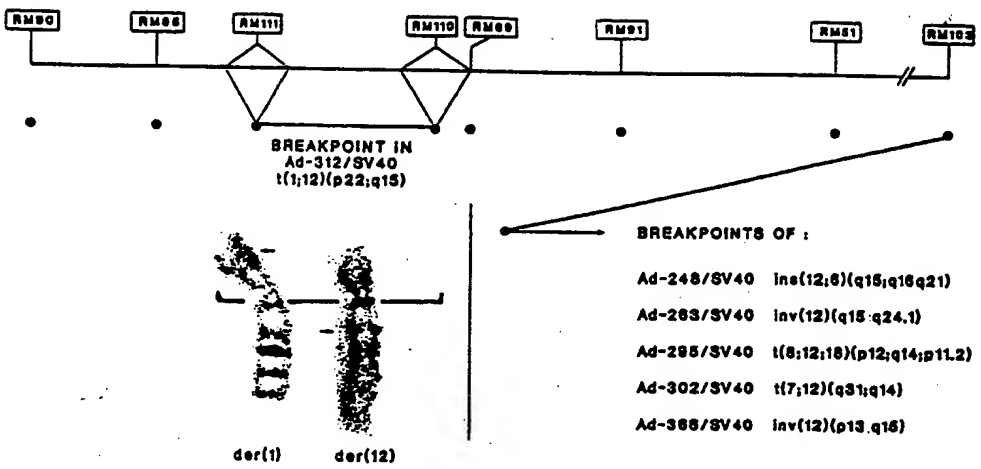


Figure 4 Schematic representation of FISH mapping data obtained for the six pleomorphic salivary gland adenoma cell lines tested in this study. The specific chromosome 12 aberrations in the various cell lines are given. Cosmid clones which were used as probes in the FISH mapping studies correspond to sequence-tagged sites obtained from overlapping YAC clones. Individual FISH experiments are indicated by dots. Cosmid clones were named after the acronyms of the STSs, as shown in the boxes, and the relative order of these is as presented. The DNA interval between RM90 and RM103 is estimated to be about 1.3 Mbp. Insert: Schematic representation of the G-banded derivative chromosomes der(1) and der(12) of the Ad-312/SV40 cell line, which carries a t(1;12)(p22;q15). The positions of the chromosome 12 breakpoints of Ad-248/SV40, Ad-263/SV40, Ad-295/SV40, Ad-302/SV40, and Ad-366/SV40 are distal to RM103 as indicated by the arrow.

YAC clone might span the chromosome 12 breakpoint in this cell line. It should be noted that G-banding revealed a telomeric association involving the short arm of chromosome 12 in Figure 2C. The observation that YAC clone Y9091 spanned the chromosome 12 breakpoint in Ad-312/SV40 was confirmed independently in FISH studies in which cosmid clone cRM90 or cRM91 was used as molecular probe; they were shown to contain the alternative end-sequences of the Y9091 insert. cRM90 appeared to map distally to the chromosome 12 breakpoint, whereas cRM91 was found to map proximally (data not shown). These results also established the chromosomal orientation of the YAC contig shown in Figure 1. In summary, we concluded from these FISH studies that the chromosome 12 translocation breakpoint in Ad-312/SV40 must be located in the DNA interval corresponding to the overlapping sequences (about 300 kbp) of the two YAC clones.

Fine Mapping of the Chromosome 12 Translocation Breakpoint of Ad-312/SV40

In an approach to further narrow the chromosome 12 translocation breakpoint region of Ad-312/SV40, cosmid clones with different mapping positions within YAC clone Y9091 were isolated. These included cRM89, cRM85, cRM110, and cRM111. cRM89 and cRM85 were isolated on the basis of STS sequences of YAC clones not shown here. cRM110 and cRM111 were obtained via inter-Alu-PCR. RM110 was shown by Southern blot analysis to hybridize to a terminal *MluI* fragment of Y9091 and not to the DNA insert of the overlapping YAC clone with RM89 as telomeric end-sequences. The location of RM110 is as indicated in Figure 1. RM111 was shown to hybridize to a *BssHII*, *MluI*, *PvuI*, and *SfiI* fragment of Y9091 and is therefore located in the PvuI-SfiI fragment of Y9091, to which STS RM48 was also mapped (Fig. 1). FISH analysis of metaphase chromosomes of Ad-312/SV40 with cRM89 or cRM110 as probe indicated that the DNA insert of these cosmids mapped proximally to the chromosome 12 translocation breakpoint in this cell line, as illustrated for cRM89 in Figure 3A. Subsequent FISH analysis of Ad-312/SV40 with cRM85 or cRM111 as probe revealed hybridization signals distally to the translocation breakpoint, as illustrated for cRM111 in Figure 3B. The results with cRM85 and cRM111 are in agreement with the observed breakpoint spanning by YAC clone Y4854, as cRM85 maps distally and cRM111 closely to STS RM48, which marks the telomeric end of the YAC clone Y4854. In conclusion, the chromosome 12 translocation breakpoint in Ad-312/SV40 must be located in the DNA interval between cRM110 and cRM111, as summarized schematically in Figure 4.

FISH Evaluation of Chromosome 12 Breakpoints in Other Pleomorphic Salivary Gland Adenoma Cell Lines

To determine the position of their chromosome 12 breakpoints relative to that of Ad-312/SV40, five other pleomorphic salivary gland adenoma cell lines were evaluated by FISH analysis, as summarized schematically in Figure 4. These cell lines, which were developed from primary tumors [5, 8], included Ad-248/SV40, Ad-263/SV40, Ad-295/SV40, Ad-302/SV40, and Ad-366/SV40. The chromosome 12 aberrations of these cell lines are listed in Figure 4. FISH analysis of metaphase chromosomes of these cell lines using cRM91 revealed that the chromosome 12 breakpoints of all these cell lines mapped proximally to this cosmid clone (data not shown). Similar FISH analysis was also performed using a cosmid clone corresponding to sequence-tagged site RM103 as a probe. RM103 was found to map proximally to RM91 at a distance of about 0.9 Mbp. In all cases, cRM103 appeared to map distally to the chromosome 12 translocation breakpoints, indicating that the chromosome 12 breakpoints in these five pleomorphic salivary gland adenoma cell lines are located at a relatively large distance from that of Ad-312/SV40 cells.

DISCUSSION

In the studies presented here, we have identified, molecularly cloned, and characterized a chromosome region on the long arm of chromosome 12 in which the translocation breakpoint of pleomorphic salivary gland adenoma cell line Ad/312/SV40 appears to map. In previous studies [5], we already provided evidence that the chromosome 12 breakpoint of this cell line was located between D12S8 and *CHOP*. Because the two breakpoints spanning YAC clones described here were obtained in directional chromosome walking experiments using D12S8 and the *CHOP* gene as initial starting points, the chromosome 12 breakpoint mapping results presented here confirm our previous claim. The FISH results obtained with the complete YAC insert of Y9091 as molecular probe were confirmed independently in FISH studies using cosmid clones containing sequences corresponding to various regions of the insert of this YAC clone. This is of importance, as the independent confirmatory results make it rather unlikely that the split signals observed with the complete insert of Y9091 can be explained otherwise than by a factual splitting of sequences represented in the YAC. The presence, for instance, of highly related genetic sequences on both sides of a chromosome breakpoint could easily lead to erroneous conclusions if they were based solely on FISH results of a YAC insert. Finally, our mapping studies have also established conclusively the chromosomal orientation of the long-range restriction map we have generated in these studies. This orientation was already predicted on the basis of two-color FISH studies (unpublished observations).

The FISH studies, described here, enabled us to map the chromosome 12 breakpoint in Ad/312/SV40 cells to the 190-kbp DNA interval between the established STSs RM48 and RM69. However, the breakpoint region can be narrowed somewhat further on the basis of the following. The fact that Y4854 was shown to span the breakpoint indicates that at least a considerable part of the telomeric half of the YAC clone must map distally to the breakpoint. Precisely how much remains to be established. On the other side, STS RM69 appeared to be located in about the middle of the DNA insert of cosmid clone cRM69, suggesting that the breakpoint is close to 25 kbp distally to RM69. Moreover, cRM69 appeared to lack RM110 (data not shown) and, as cRM110 was found proximally to the chromosome 12 breakpoint in Ad/312/SV40 cells, the breakpoint should be even further distal to RM69 than the earlier-mentioned 25 kbp. Altogether, this narrows the chromosome 12 breakpoint region to a DNA interval, which must be considerably smaller than 165 kbp. Further pinpointing of the breakpoint will allow us to molecularly clone the chromosome 12 breakpoint and to characterize the genetic sequences in the breakpoint junction region, which might lead to the identification of pathogenetically relevant sequences. Identification of the genes present in the DNA inserts of YAC clones Y4854 and Y9091, via sequencing, direct hybridization, direct selection, or exon-trapping, might constitute a useful alternative approach for identifying the gene in this region of the long arm of chromosome 12 that might be pathogenetically critical for pleomorphic salivary gland adenoma tumorigenesis.

The observation that the chromosome 12 breakpoints in other pleomorphic salivary gland adenomas are located in a remote and more proximal region on the long arm of chromosome 12 is of interest. It could imply that the chromosome 12 breakpoints in pleomorphic salivary gland adenomas are dispersed over a relatively large DNA region of the long arm of chromosome 12, reminiscent of the 11q13 breakpoints in B-cell malignancies [22]. Elucidation of the precise location of the chromosome 12 breakpoints in the other pleomorphic salivary gland adenoma cell lines could shed more light on this matter. On the other hand, it could point towards alternative sequences on the long arm of chromosome 12 between D12S8 and the *CHOP* gene that might be of importance, presumably for growth regulation in pleomorphic salivary gland adenoma. The fact that the chromosome 12 breakpoint region described here has so far been found only in the Ad-312/SV40 cell line makes it necessary to analyze a larger number of salivary gland adenomas with chromosome 12q13–q15 aberrations to assess the potential relevance for tumorigenesis of the chromosome 12 sequences affected in the studied cell line. If more cases with aberrations in this particular region of chromosome 12 can be found, it would be of interest to find out whether these tumors form a clinical subgroup. Finally, chromosome translocations involving region q13–q15 of human chromosome 12 have been reported for a variety of other solid tumors: benign adipose tissue tumors, uterine leiomyoma, rhabdomyosarcoma, hemangiopericytoma, clear-cell sarcoma, chondromatous tumors, and hamartoma of the lung. Whether or not the chromosome 12 breakpoints in some of these tumors map within the same region as that of Ad-312/SV40 remains to be established. The YAC and cosmid clones described in this report constitute useful tools for investigating this.

The availability of a copy of the first-generation CEPH YAC library [10] and a copy of the arrayed chromosome-12-specific cosmid library (LLNL12NC01) [11] is greatly acknowledged. The cosmid library was

constructed as part of the National Laboratory Gene Library Project under the auspices of the U.S. DOE by LLNL under contract No. W-7405-Eng-48. The authors acknowledge the excellent technical assistance of M. Debasen, C. Huysmans, E. Mayen, K. Meyer-Bolte, and M. Willems and would like to thank M. Leys for artwork. This work was supported in part by the EC through Biomed 1 program "Molecular Cytogenetics of Solid Tumours," the "Geconcerteerde Onderzoekacties 1992–1996," the "Association Luxembourgeoise contre le Cancer," the National Fund for Scientific Research (NFWO; Kom op tegen Kanker), the "ASLK-programma voor Kankeronderzoek," the "Schwerpunktprogramm: Molekulare und Klassische Tumorcytogenetik" of the Deutsche Forschungsgemeinschaft, and the Tönjes-Vagt Stiftung. This text presents results of the Belgian program on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming. The scientific responsibility is assumed by its authors. J.W.M. Geurts is an Aspirant of the National Fund for Scientific Research (NFWO; Kom op tegen Kanker).

REFERENCES

1. Seifert G, Miehlke A, Haubrich J, Chilla R (1986): Diseases of the salivary glands. Pathology Diagnosis. Treatment. Facial nerve surgery. (Translated by P.M. Stell.) Thieme, Stuttgart, New York, pp. 182–194.
2. Sandros J, Stenman G, Mark J (1990): Cytogenetic and molecular observations in human and experimental salivary gland tumours. Cancer Genet Cytogenet 44:153–187.
3. Bullerdiek J, Wobst G, Meyer-Bolte K, Chilla R, Haubrich J, Thode B, Bartnitzke S (1993): Cytogenetic subtyping of 220 salivary gland pleomorphic adenomas: correlation to occurrence, histological subtype, and in vitro cellular behavior. Cancer Genet Cytogenet 65:27–31.
4. Schoenmakers HFPM, Kools PFJ, Kazmierczak B, Bullerdiek J, Claussen U, Horsthemke B, Van den Berghe H, Van de Ven WJM (1993): Isolation of a somatic cell hybrid retaining the der(18) t(12;18)(q13;p11.2) from a myxoid liposarcoma cell line. Cancer Genet Cytogenet 67:159–161.
5. Schoenmakers HFPM, Kools PFJ, Mols R, Kazmierczak B, Bartnitzke S, Bullerdiek J, Dal Cin P, De Jong PJ, Van den Berghe H, Van de Ven WJM (1993): Physical mapping of chromosome 12q breakpoints in lipoma, pleomorphic salivary gland adenoma, uterine leiomyoma, and myxoid liposarcoma. Genomic 20:210–222.
6. Keats B, Ott J, Connealy M (1989): Reports of the committee on linkage and gene order. Cytogenet Cell Genet 51:459–502.
7. Stenman G, Sahlin P, Mark J, Chaganti RKS, Kladblom LS, Aman P (1993): The 12q13–q15 translocation breakpoints in pleomorphic adenoma and clear-cell sarcoma of tendons and aponeuroses are different from that in myxoid liposarcoma. Genes Chrom Cancer 7:178–180.
8. Kazmierczak B, Bartnitzke S, Harti M, Bullerdiek J (1990): In vitro transformation by the SV40 "early region" of cells from a human benign salivary gland tumour with a 12q13→q15 rearrangement. Cytogenet Cell Genet 53:37–39.
9. Warburton D, Gersen S, Yu M-T, Jackson C, Handelin B, Housman D (1990): Monochromosomal rodent-human hybrids from microcell fusion of human lymphoblastoid cells containing an inserted dominant selectable marker. Genomics 8:358–366.
10. Albertsen HM, Abderrahim H, Cann HM, Dausset J, Le Paslier D, Cohen D (1990): Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. Proc Natl Acad Sci USA 87:4256–4260.
11. Montgomery KT, LeBlanc JM, Tsai P, McNinch JS, Ward DC, De Jong PJ, Kucherlapati R, Krauter KS (1993): Characterization of two chromosome 12 cosmid libraries and development of STSs from cosmids mapped by FISH. Genomics 17:682–693.
12. Green ED, Olson MV (1990): Systematic screening of yeast artificial-chromosome libraries using the polymerase chain reaction. Proc Natl Acad Sci USA 87:1213–1217.
13. Geurts JMW, Schoenmakers HFPM, Mols R, Van de Ven WJM (1994): Improved procedure for rapid isolation and sequencing of DNA termini in yeast artificial chromosomes. Meth Mol Cell Biol 4:257–265.
14. Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT (1989): Alu polymerase chain reaction. A method for rapid isolation of human-specific sequences from complex DNA sources. Proc Natl Acad Sci USA 86:6686–6690.
15. Breukel C, Wijnen J, Trops C, Van de Klift H, Dauwerse H, Meera Khan P (1990): Vector-Alu PCR: a rapid step in mapping cosmids and YACs. Nucl Acids Res 18:3087.
16. Sambrook J, Fritsch EF, Maniatis T (1989): Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
17. Chu G, Vollrath D, Davis RW (1986): Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science 234:1582–1585.
18. Smit VTHBM, Wessels JW, Mollevanger P, Schrier PI, Raap KK, Beverstock GC, Cornelisse CJ (1990): Combined GTG-banding and nonradioactive in situ hybridization improves characterization of complex karyotypes. Cytogenet Cell Genet 54:20–23.
19. Kievits T, Dauwerse JG, Wiegant J, Devilee P, Breuning MH, Cornelisse CJ, van Ommen G, Pearson PL (1990): Rapid subchromosomal localization of cosmids by nonradioactive in situ hybridization. Cytogenet Cell Genet 53:134–136.
20. Kools PFJ, Roebroek AJM, Van de Velde HJK, Marynen P, Bullerdiek J, Van de Ven WJM (1993): Regional mapping of the human *NSP* gene to chromosome 14q21–q22 by fluorescence in situ hybridization. Cytogenet Cell Genet 66:48–50.
21. Looijenga LHJ, Smit VTHBM, Wessels JW, Mollevanger P, Oosterhuis JW, Cornelisse CJ (1990): Localization and polymorphism of a chromosome 12-specific α satellite DNA sequence. Cytogenet Cell Genet 53:215–218.
22. Raynaud SD, Bekri S, Leroux D, Grosgeorge J, Klein B, Bastard C, Gaudray P, Simon MP (1993): Expanded range of 11q13 breakpoints with differing patterns of cyclin D1 expression in B-cell malignancies. Genes Chrom Cancer 8:80–87.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: K.U. Leuven Research & Development
(B) STREET: Benedenstraat 59
(C) CITY: Leuven
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-3000
(G) TELEPHONE: --
(H) TELEFAX: --
(I) TELEX: --

(ii) TITLE OF INVENTION: Multiple-tumor aberrant growth genes

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 95201951.1

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1033 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: lipoma
(H) CELL LINE: Li-501/SV40

(vii) IMMEDIATE SOURCE:
(B) CLONE: pCH111

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 12+3
(B) MAP POSITION: 12q15+3q27-q28

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..1031
(D) OTHER INFORMATION: /partial
/c don_start= 3
/product= "fusion gene HMG and LPP"
/label= HMG-LPP

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..182
(D) OTHER INFORMATION: /partial
/codon_start= 3
/product= "part of High Mobility Group protein"
/label= HMG

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 183..1031
(D) OTHER INFORMATION: /partial
/codon_start= 183
/product= "part of Lipoma-Preferred Partner gene"
/label= LPP

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..18

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1000..1033

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CG CTT CAG AAG AGA GGA CGC GGC CGC CCC AGG AAG CAG CAG CAA AAA      47
   Leu Gln Lys Arg Gly Arg Gly Arg Pro Arg Lys Gln Gln Gln Lys
     1               5                  10                  15

CCA ACC GGT GAG CCC TCT CCT AAG AGA CCC AGG GGA AGA CCC AAA GGC      95
Pro Thr Gly Glu Pro Ser Pro Lys Arg Pro Arg Gly Arg Pro Lys Gly
                20                  25                  30

AGC AAA AAC AAG AGT CCC TCT AAA GCA GCT CAA GAG GAA GCA GAA GCC     143
Ser Lys Asn Lys Ser Pro Ser Lys Ala Ala Gln Glu Glu Ala Glu Ala
             35                  40                  45

ACT GAA GAA AAA CGG CCA AGG GGC AGA CCT AGG AAA TGG GGT GGC CAT     191
Thr Glu Glu Lys Arg Pro Arg Gly Arg Pro Arg Lys Trp Gly Gly His
          50                  55                  60

TCA GGG CAA CTG GGG CCT TCG TCA GTT GCC CCT TCA TTC CGC CCA GAG     239
Ser Gly Gln Leu Gly Pro Ser Ser Val Ala Pro Ser Phe Arg Pro Glu
      65                  70                  75

GAT GAG CTT GAG CAC CTG ACC AAA AAG ATG CTG TAT GAC ATG GAA AAT     287
Asp Glu Leu Glu His Leu Thr Lys Lys Met Leu Tyr Asp Met Glu Asn
  80                  85                  90                  95
```

```
CCA CCT GCT GAC GAA TAC TTT GGC CGC TGT GCT CGC TGT GGA GAA AAC   335
Pro Pro Ala Asp Glu Tyr Phe Gly Arg Cys Ala Arg Cys Gly Glu Asn
                100                 105                 110

GTA GTT GGG GAA GGT ACA GGA TGC ACT GCC ATG GAT CAG GTC TTC CAC   383
Val Val Gly Glu Gly Thr Gly Cys Thr Ala Met Asp Gln Val Phe His
            115                 120                 125

GTG GAT TGT TTT ACC TGC ATC ATC TGC AAC AAC AAG CTC CGA GGG CAG   431
Val Asp Cys Phe Thr Cys Ile Ile Cys Asn Asn Lys Leu Arg Gly Gln
        130                 135                 140

CCA TTC TAT GCT GTG GAA AAG AAA GCA TAC TGC GAG CCC TGC TAC ATT   479
Pro Phe Tyr Ala Val Glu Lys Lys Ala Tyr Cys Glu Pro Cys Tyr Ile
    145                 150                 155

AAT ACT CTG GAG CAG TGC AAT GTG TGT TCC AAG CCC ATC ATG GAG CGG   527
Asn Thr Leu Glu Gln Cys Asn Val Cys Ser Lys Pro Ile Met Glu Arg
160                 165                 170                 175

ATT CTC CGA GCC ACC GGG AAG GCC TAT CAT CCT CAC TGT TTC ACC TGC   575
Ile Leu Arg Ala Thr Gly Lys Ala Tyr His Pro His Cys Phe Thr Cys
                180                 185                 190

GTG ATG TGC CAC CGC AGC CTG GAT GGG ATC CCA TTC ACT GTG GAT GCT   623
Val Met Cys His Arg Ser Leu Asp Gly Ile Pro Phe Thr Val Asp Ala
            195                 200                 205

GGC GGG CTC ATT CAC TGC ATT GAG GAC TTC CAC AAG AAA TTT GCC CCG   671
Gly Gly Leu Ile His Cys Ile Glu Asp Phe His Lys Lys Phe Ala Pro
        210                 215                 220

CGG TGT TCT GTG TGC AAG GAG CCT ATT ATG CCA GCC CCG GGC CAG GAG   719
Arg Cys Ser Val Cys Lys Glu Pro Ile Met Pro Ala Pro Gly Gln Glu
    225                 230                 235

GAG ACT GTC CGT ATT GTG GCT TTG GAT CGA GAT TTC CAT GTT CAC TGC   767
Glu Thr Val Arg Ile Val Ala Leu Asp Arg Asp Phe His Val His Cys
240                 245                 250                 255

TAC CGA TGC GAG GAT TGC GGT GGT CTC CTG TCT GAA GGA GAT AAC CAA   815
Tyr Arg Cys Glu Asp Cys Gly Gly Leu Leu Ser Glu Gly Asp Asn Gln
                260                 265                 270

GGC TGC TAC CCC TTG GAT GGG CAC ATC CTC TGC AAG ACC TGC AAC TCT   863
Gly Cys Tyr Pro Leu Asp Gly His Ile Leu Cys Lys Thr Cys Asn Ser
            275                 280                 285

GCC CGC ATC AGG GTG TTG ACC GCC AAG GCG AGC ACT GAC CTT TAG ATT   911
Ala Arg Ile Arg Val Leu Thr Ala Lys Ala Ser Thr Asp Leu  *  Ile
        290                 295                 300

CAG TCA CCT GTT CAG CCG GCA CTG AGA AGA ACG AAC ACA AGA AAA AGA   959
Gln Ser Pro Val Gln Pro Ala Leu Arg Arg Thr Asn Thr Arg Lys Arg
    305                 310                 315
```

```
TAA GAA ATA CTA GAG TAA AGG CCA TCA AAC TAC GCG AAA AAA AAA AAA   1007
 *  Glu Ile Leu Glu  *  Arg Pro Ser Asn Tyr Ala Lys Lys Lys Lys
320                 325                 330                 335

AAA AAA AAA GAT GTC GAC GGA TCC TT                              1033
Lys Lys Lys Asp Val Asp Gly Ser
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4323 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: small intestine

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: small intestine cDNA library (Clontech, cat. no. HL1133b)
(B) CLONE: various clones were used

(viii) POSITION IN GENOME:
(B) MAP POSITION: 3q27-28

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 247..2085

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
GTCACTTTTA TTTGGGGGTG TGGACAGCTG CTTTCCCAGG GGAGTACTTC TTACAGTGGG     60

ATTTCAAGAC AAGATCGGCC TGAAGAAAAA TTATATTTGT ATATTTTTTA AAAAGTGGGA    120

ACTTTGAGGC TCAGAGACAG AGCAGAAGAC AGAACCTGGT CTTCTGATTC CCTGTGTTCT    180

GCTTTTTTCA TTGTTCCACT GGACGCTCAT CAGAGGGAAG ATCTTTTTCC TCAATTGATT    240

CCAACA ATG TCT CAC CCA TCT TGG CTG CCA CCC AAA AGC ACT GGT GAG         288
       Met Ser His Pro Ser Trp Leu Pro Pro Lys Ser Thr Gly Glu
         1               5                  10

CCC CTC GGC CAT GTG CCT GCA CGG ATG GAG ACC ACC CAT TCC TTT GGG       336
Pro Leu Gly His Val Pro Ala Arg Met Glu Thr Thr His Ser Phe Gly
 15                  20                  25                  30

AAC CCC AGC ATT TCA GTG TCT ACA CAA CAG CCA CCC AAA AAG TTT GCC       384
Asn Pro Ser Ile Ser Val Ser Thr Gln Gln Pro Pro Lys Lys Phe Ala
                 35                  40                  45

CCG GTA GTT GCT CCA AAA CCT AAG TAC AAC CCA TAC AAA CAA CCT GGA       432
Pro Val Val Ala Pro Lys Pro Lys Tyr Asn Pro Tyr Lys Gln Pro Gly
             50                  55                  60
```

```
GGT GAG GGT GAT TTT CTT CCA CCC CCA CCT CCA CCT CTA GAT GAT TCC      480
Gly Glu Gly Asp Phe Leu Pro Pro Pro Pro Pro Pro Leu Asp Asp Ser
          65                  70                  75

AGT GCC CTT CCA TCT ATC TCT GGA AAC TTT CCT CCT CCA CCA CCT CTT      528
Ser Ala Leu Pro Ser Ile Ser Gly Asn Phe Pro Pro Pro Pro Pro Leu
      80                  85                  90

GAT GAA GAG GCT TTC AAA GTA CAG GGG AAT CCC GGA GGC AAG ACA CTT      576
Asp Glu Glu Ala Phe Lys Val Gln Gly Asn Pro Gly Gly Lys Thr Leu
 95                  100                 105                 110

GAG GAG AGG CGC TCC AGC CTG GAC GCT GAG ATT GAC TCC TTG ACC AGC      624
Glu Glu Arg Arg Ser Ser Leu Asp Ala Glu Ile Asp Ser Leu Thr Ser
                115                 120                 125

ATC TTG GCT GAC CTT GAG TGC AGC TCC CCC TAT AAG CCT CGG CCT CCA      672
Ile Leu Ala Asp Leu Glu Cys Ser Ser Pro Tyr Lys Pro Arg Pro Pro
            130                 135                 140

CAG AGC TCC ACT GGT TCA ACA GCC TCT CCT CCA GTT TCG ACC CCA GTC      720
Gln Ser Ser Thr Gly Ser Thr Ala Ser Pro Pro Val Ser Thr Pro Val
        145                 150                 155

ACA GGA CAC AAG AGA ATG GTC ATC CCG AAC CAA CCC CCT CTA ACA GCA      768
Thr Gly His Lys Arg Met Val Ile Pro Asn Gln Pro Pro Leu Thr Ala
    160                 165                 170

ACC AAG AAG TCT ACA TTG AAA CCA CAG CCT GCA CCC CAG GCT GGA CCC      816
Thr Lys Lys Ser Thr Leu Lys Pro Gln Pro Ala Pro Gln Ala Gly Pro
175                 180                 185                 190

ATC CCT GTG GCT CCA ATC GGA ACA CTC AAA CCC CAG CCT CAG CCA GTC      864
Ile Pro Val Ala Pro Ile Gly Thr Leu Lys Pro Gln Pro Gln Pro Val
                195                 200                 205

CCA GCC TCC TAC ACC ACG GCC TCC ACT TCT TCA AGG CCT ACC TTT AAT      912
Pro Ala Ser Tyr Thr Thr Ala Ser Thr Ser Ser Arg Pro Thr Phe Asn
            210                 215                 220

GTG CAG GTG AAG TCA GCC CAG CCC AGC CCT CAT TAT ATG GCT GCC CCT      960
Val Gln Val Lys Ser Ala Gln Pro Ser Pro His Tyr Met Ala Ala Pro
        225                 230                 235

TCA TCA GGA CAA ATT TAT GGC TCA GGG CCC CAG GGC TAT AAC ACT CAG     1008
Ser Ser Gly Gln Ile Tyr Gly Ser Gly Pro Gln Gly Tyr Asn Thr Gln
    240                 245                 250

CCA GTT CCT GTC TCT GGG CAG TGT CCA CCT CCT TCA ACA CGG GGA GGC     1056
Pro Val Pro Val Ser Gly Gln Cys Pro Pro Pro Ser Thr Arg Gly Gly
255                 260                 265                 270

ATG GAT TAT GCC TAC ATT CCA CCA CCA GGA CTT CAG CCG GAG CCT GGG     1104
Met Asp Tyr Ala Tyr Ile Pro Pro Pro Gly Leu Gln Pro Glu Pro Gly
                275                 280                 285
```

```
TAT GGG TAT GCC CCC AAC CAG GGA CGC TAT TAT GAA GGC TAC TAT GCA    1152
Tyr Gly Tyr Ala Pro Asn Gln Gly Arg Tyr Tyr Glu Gly Tyr Tyr Ala
            290                 295                 300

GCA GGG CCA GGC TAT GGG GGC AGA AAT GAC TCT GAC CCT ACC TAT GGT    1200
Ala Gly Pro Gly Tyr Gly Gly Arg Asn Asp Ser Asp Pro Thr Tyr Gly
        305                 310                 315

CAA CAA GGT CAC CCA AAT ACC TGG AAA CGG GAA CCA GGG TAC ACT CCT    1248
Gln Gln Gly His Pro Asn Thr Trp Lys Arg Glu Pro Gly Tyr Thr Pro
    320                 325                 330

CCT GGA GCA GGG AAC CAG AAC CCT CCT GGG ATG TAT CCA GTC ACT GGT    1296
Pro Gly Ala Gly Asn Gln Asn Pro Pro Gly Met Tyr Pro Val Thr Gly
335                 340                 345                 350

CCC AAG AAG ACC TAT ATC ACA GAT CCT GTT TCA GCC CCC TGT GCG CCA    1344
Pro Lys Lys Thr Tyr Ile Thr Asp Pro Val Ser Ala Pro Cys Ala Pro
                355                 360                 365

CCA TTG CAG CCA AAG GGT GGC CAT TCA GGG CAA CTG GGG CCT TCG TCA    1392
Pro Leu Gln Pro Lys Gly Gly His Ser Gly Gln Leu Gly Pro Ser Ser
            370                 375                 380

GTT GCC CCT TCA TTC CGC CCA GAG GAT GAG CTT GAG CAC CTG ACC AAA    1440
Val Ala Pro Ser Phe Arg Pro Glu Asp Glu Leu Glu His Leu Thr Lys
        385                 390                 395

AAG ATG CTG TAT GAC ATG GAA AAT CCA CCT GCT GAC GAA TAC TTT GGC    1488
Lys Met Leu Tyr Asp Met Glu Asn Pro Pro Ala Asp Glu Tyr Phe Gly
    400                 405                 410

CGC TGT GCT CGC TGT GGA GAA AAC GTA GTT GGG GAA GGT ACA GGA TGC    1536
Arg Cys Ala Arg Cys Gly Glu Asn Val Val Gly Glu Gly Thr Gly Cys
415                 420                 425                 430

ACT GCC ATG GAT CAG GTC TTC CAC GTG GAT TGT TTT ACC TGC ATC ATC    1584
Thr Ala Met Asp Gln Val Phe His Val Asp Cys Phe Thr Cys Ile Ile
                435                 440                 445

TGC AAC AAC AAG CTC CGA GGG CAG CCA TTC TAT GCT GTG GAA AAG AAA    1632
Cys Asn Asn Lys Leu Arg Gly Gln Pro Phe Tyr Ala Val Glu Lys Lys
            450                 455                 460

GCA TAC TGC GAG CCC TGC TAC ATT AAT ACT CTG GAG CAG TGC AAT GTG    1680
Ala Tyr Cys Glu Pro Cys Tyr Ile Asn Thr Leu Glu Gln Cys Asn Val
        465                 470                 475

TGT TCC AAG CCC ATC ATG GAG CGG ATT CTC CGA GCC ACC GGG AAG GCC    1728
Cys Ser Lys Pro Ile Met Glu Arg Ile Leu Arg Ala Thr Gly Lys Ala
    480                 485                 490

TAT CAT CCT CAC TGT TTC ACC TGC GTG ATG TGC CAC CGC AGC CTG GAT    1776
Tyr His Pro His Cys Phe Thr Cys Val Met Cys His Arg Ser Leu Asp
495                 500                 505                 510
```

```
GGG ATC CCA TTC ACT GTG GAT GCT GGC GGG CTC ATT CAC TGC ATT GAG    1824

Gly Ile Pro Phe Thr Val Asp Ala Gly Gly Leu Ile His Cys Ile Glu
                515                 520                 525

GAC TTC CAC AAG AAA TTT GCC CCG CGG TGT TCT GTG TGC AAG GAG CCT    1872
Asp Phe His Lys Lys Phe Ala Pro Arg Cys Ser Val Cys Lys Glu Pro
            530                 535                 540

ATT ATG CCA GCC CCG GGC CAG GAG GAG ACT GTC CGT ATT GTG GCT TTG    1920
Ile Met Pro Ala Pro Gly Gln Glu Glu Thr Val Arg Ile Val Ala Leu
        545                 550                 555

GAT CGA GAT TTC CAT GTT CAC TGC TAC CGA TGC GAG GAT TGC GGT GGT    1968
Asp Arg Asp Phe His Val His Cys Tyr Arg Cys Glu Asp Cys Gly Gly
    560                 565                 570

CTC CTG TCT GAA GGA GAT AAC CAA GGC TGC TAC CCC TTG GAT GGG CAC    2016
Leu Leu Ser Glu Gly Asp Asn Gln Gly Cys Tyr Pro Leu Asp Gly His
575                 580                 585                 590

ATC CTC TGC AAG ACC TGC AAC TCT GCC CGC ATC AGG GTG TTG ACC GCC    2064
Ile Leu Cys Lys Thr Cys Asn Ser Ala Arg Ile Arg Val Leu Thr Ala
                595                 600                 605

AAG GCG AGC ACT GAC CTT TAGATTCAGT CACCTGTTCA GCCGGCACTG           2112
Lys Ala Ser Thr Asp Leu
            610

AGAAGAACGA ACACAAGAAA AAGATAAGAA ATACTAGAGT AAAGGCCATC AAACTACGCG  2172

ATAGTCTCTG TTCTTCATCT GCTATTAACC TTGCCTTAGA AACACATAAA TTATGAGATT  2232

TTTTTTTAAA AGTTGTTACC AAATACACAT TTCACATTGA ATCATGTAGG ATCTTGATGG  2292

GCCTTTGTTC CCAAGGACTT CCACATTTTT GCACAGATTA TGCTCCATCC CTTCACTTCT  2352

GCATTCCTGT AACTTTTAAT CCCTATGTTT GTCTCACTTT TCATCTGGTT GAATGGCTTT  2412

TCTTAGTGTG GTATTTGCTG TCACATAGTT TTTTCCTGGG TGAGTCTGCC AACTCACAGG  2472

TGCTTTTAGG CTTGAAATCT CCATCCTATC ATTTCCGTTT TGCCTGTGAC TGTAAAGAGT  2532

AGCCATTCTT TTCCCATGTA TTGAAGAGGA TATTCTTCTC TTGCTTTATA CTACTCACGT  2592

CCTTGGGGAG GGAAATGCAC AATTTTTTTT TGTTAGGCTG TAAAGAATTT AAGCTGTAAA  2652

TTACATAAGT TAGAACAAGC CCAAATTTAA TTTGCAACCA TCAGAATTCA GAATCTATAG  2712

TGACCAGTGA TCAAGGCTAA TTGGAAAAGA GTTATCGGCC CATAGCTAAT AAGTAGTGAC  2772

AGACAACCAA GCTTCAATAT TTTTCTAAAG AAATTACAGG TGGGATATGC TAGAAAAGGC  2832

ATTTTGGGGT TATGTTTAAA AAAACATTAT TGTCCCACAA TATTACCTTA AGATTTTTCT  2892

TTTCCGCACT ACCTGAACAT TGTAATACAG ACAAACTTGA TTTCTTCTAG AAGATAACAT  2952

TTTCAATACT GTCCCACTTC TCATCTTAAA AATATTGTCA TGTTTATTCT AATATCCAAC  3012
```

GCAACTATCA AAATTGCCTT TTTCTCTAGA GGATGAAGGC TGTGAAAAAA CCGTTCAAAT 3072

TCTCTTCTTT TTCTTTTTTA TTACCAGGTC CATTTTGCCT GACAATTGCA AATCAGAGCA 3132

TACAAAATAA AACTGTGCAG TTTTGTTTGG TTTACTTTCA AAAGAGTAGA AAGCTTGAAA 3192

AGATTCTGAA ACCACAGTTT CATTATTCTC ATAATCCTTC TGCAACTGAA ATTACATATT 3252

GCAGGAGACA TTTTCATATC ATCAATGTGA CATTTACACC ACACTTTCAA AGACAATCAC 3312

TGAAACAAAA ATTGTCTTTA TGAGCTAAAA ATATGCAGAA TCTCTGCCTA GAATCTTTAT 3372

TCAAACTTTT ATTAGCCAGT GAAACACTTG CTTGCCAACT GCCAAGCCAT ACTTATTAAG 3432

TTCGAACATG TTTCACTTAA GGAGAGACAC CTAGCTTAGT CATGGCAAGT TGCCATTTTG 3492

TAAACTAAGG ATTTTGGACT GAGATTTCTT AAATCTTTCT TCAAATCTCC CACAAGTATA 3552

TACTTTTAAA TTATGGAGTA TTTTAAGTCT ACAAAAAGGT ATAAATAATA ATATAATGAA 3612

TTCCTATATA CCTAATACCC AGTTTAAGAC ACCAAATATA ACAAGTATAA TTACATCCTC 3672

CAATGTACCG TTTCCTTATT CCACAGATAT CTTTTTCATT ATTGTGAAGT GATGTTCAGA 3732

TTTCTAGTTT TTTTTTCTAG TTTTTAATTT TAACATCAGA ACTGAAATAA AAAATTATGG 3792

ATACGTGTTT TGAATTGCAA ACTATTCCTC AGGAATTCCA ATTAAATTTA TTTTACTTGA 3852

ATAGGAATGA TCATAAAAGT GATTCTTTTT TTGTGACTAG AAATTCTTAA GCCGATGGTC 3912

ACTATAGCTC ATCCTTAATG TATGGCTCAT TTGCTTTTGT CACTAAACGG TTTTGTGTTA 3972

GAACCACCAA AATTATAGCT TTTAAGAGCT TCCTTTGACC ACTGTCTTTT TCTTACCCTA 4032

CTTCTCTTAT CTTTGATCGT ATATTTCTCA TAATGTGAAA TATGATGAGA TTCACTTAGG 4092

GGCAGCATGT TAGTTTTGGG AGGCAATGTC AACTGTGTCT CTGAATTCCT GTCTTCCAAA 4152

TTGAAGCCAG ACCATGCTGA TGACCTCAAG TAGCACTGAC TATTTGACAA TAGGGCTGAT 4212

AATGTAATCG GCTTGAATTT TGACTTAGTA ACTTTTTATG TAATACTTTC GGAGAAATTC 4272

TCTTTAGGAC AAAGCAGAGA GTCCAATTTA TTGAGGGATA GATTGTATCT C 4323

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 612 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: small intestine

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 413..470
(D) OTHER INFORMATION: /label= LIM1
/note= "first LIM domain of LPP gene product"

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 474..528
(D) OTHER INFORMATION: /label= LIM2
/note= "second LIM domain of LPP gene product"

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 533..598
(D) OTHER INFORMATION: /label= LIM3
/note= "third LIM domain of LPP gene product"

(ix) FEATURE:
(A) NAME/KEY: Cleavage-site
(B) LOCATION: 370..371
(D) OTHER INFORMATION: /label= BREAK-POINT
/note= "breaking-point within the LPP gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Met Ser His Pro Ser Trp Leu Pro Pro Lys Ser Thr Gly Glu Pro Leu
1               5                  10                  15

Gly His Val Pro Ala Arg Met Glu Thr Thr His Ser Phe Gly Asn Pro
            20                  25                  30

Ser Ile Ser Val Ser Thr Gln Gln Pro Pro Lys Lys Phe Ala Pro Val
        35                  40                  45

Val Ala Pro Lys Pro Lys Tyr Asn Pro Tyr Lys Gln Pro Gly Gly Glu
    50                  55                  60

Gly Asp Phe Leu Pro Pro Pro Pro Pro Pro Leu Asp Asp Ser Ser Ala
65                  70                  75                  80
```

```
Leu Pr  Ser Ile Ser Gly Asn Phe Pro Pro Pro Pro Leu Asp Glu
                85                  90                  95

Glu Ala Phe Lys Val Gln Gly Asn Pro Gly Gly Lys Thr Leu Glu Glu
            100                 105                 110

Arg Arg Ser Ser Leu Asp Ala Glu Ile Asp Ser Leu Thr Ser Ile Leu
        115                 120                 125

Ala Asp Leu Glu Cys Ser Ser Pro Tyr Lys Pro Arg Pro Pro Gln Ser
    130                 135                 140

Ser Thr Gly Ser Thr Ala Ser Pro Pro Val Ser Thr Pro Val Thr Gly
145                 150                 155                 160

His Lys Arg Met Val Ile Pro Asn Gln Pro Pro Leu Thr Ala Thr Lys
                165                 170                 175

Lys Ser Thr Leu Lys Pro Gln Pro Ala Pro Gln Ala Gly Pro Ile Pro
            180                 185                 190

Val Ala Pro Ile Gly Thr Leu Lys Pro Gln Pro Gln Pro Val Pro Ala
        195                 200                 205

Ser Tyr Thr Thr Ala Ser Thr Ser Ser Arg Pro Thr Phe Asn Val Gln
    210                 215                 220

Val Lys Ser Ala Gln Pro Ser Pro His Tyr Met Ala Ala Pro Ser Ser
225                 230                 235                 240

Gly Gln Ile Tyr Gly Ser Gly Pro Gln Gly Tyr Asn Thr Gln Pro Val
                245                 250                 255

Pro Val Ser Gly Gln Cys Pro Pro Pro Ser Thr Arg Gly Gly Met Asp
            260                 265                 270

Tyr Ala Tyr Ile Pro Pro Pro Gly Leu Gln Pro Glu Pro Gly Tyr Gly
        275                 280                 285

Tyr Ala Pro Asn Gln Gly Arg Tyr Tyr Glu Gly Tyr Tyr Ala Ala Gly
    290                 295                 300

Pro Gly Tyr Gly Gly Arg Asn Asp Ser Asp Pro Thr Tyr Gly Gln Gln
305                 310                 315                 320

Gly His Pro Asn Thr Trp Lys Arg Glu Pro Gly Tyr Thr Pro Pro Gly
                325                 330                 335

Ala Gly Asn Gln Asn Pro Pro Gly Met Tyr Pro Val Thr Gly Pro Lys
            340                 345                 350

Lys Thr Tyr Ile Thr Asp Pro Val Ser Ala Pro Cys Ala Pro Pro Leu
        355                 360                 365

Gln Pro Lys Gly Gly His Ser Gly Gln Leu Gly Pro Ser Ser Val Ala
    370                 375                 380
```

```
Pro Ser Phe Arg Pro Glu Asp Glu Leu Glu His Leu Thr Lys Lys Met
385                 390                 395                 400

Leu Tyr Asp Met Glu Asn Pro Pro Ala Asp Glu Tyr Phe Gly Arg Cys
                405                 410                 415

Ala Arg Cys Gly Glu Asn Val Val Gly Glu Gly Thr Gly Cys Thr Ala
            420                 425                 430

Met Asp Gln Val Phe His Val Asp Cys Phe Thr Cys Ile Ile Cys Asn
        435                 440                 445

Asn Lys Leu Arg Gly Gln Pro Phe Tyr Ala Val Glu Lys Lys Ala Tyr
    450                 455                 460

Cys Glu Pro Cys Tyr Ile Asn Thr Leu Glu Gln Cys Asn Val Cys Ser
465                 470                 475                 480

Lys Pro Ile Met Glu Arg Ile Leu Arg Ala Thr Gly Lys Ala Tyr His
                485                 490                 495

Pro His Cys Phe Thr Cys Val Met Cys His Arg Ser Leu Asp Gly Ile
            500                 505                 510

Pro Phe Thr Val Asp Ala Gly Gly Leu Ile His Cys Ile Glu Asp Phe
        515                 520                 525

His Lys Lys Phe Ala Pro Arg Cys Ser Val Cys Lys Glu Pro Ile Met
    530                 535                 540

Pro Ala Pro Gly Gln Glu Glu Thr Val Arg Ile Val Ala Leu Asp Arg
545                 550                 555                 560

Asp Phe His Val His Cys Tyr Arg Cys Glu Asp Cys Gly Gly Leu Leu
                565                 570                 575

Ser Glu Gly Asp Asn Gln Gly Cys Tyr Pro Leu Asp Gly His Ile Leu
            580                 585                 590

Cys Lys Thr Cys Asn Ser Ala Arg Ile Arg Val Leu Thr Ala Lys Ala
        595                 600                 605

Ser Thr Asp Leu
    610
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4067 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: YES

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 12
(B) MAP POSITION: 12q15

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 812..1141
(D) OTHER INFORMATION: /codon_start= 812
/product= "HMGI-C"
/label= HMGI-C

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTTGAATCTT GGGGCAGGAA CTCAGAAAAC TTCCAGCCCG GGCAGCGCGC GCTTGGTGCA 60

AGACTCAGGA GCTAGCAGCC CGTCCCCCTC CGACTCTCCG GTGCCGCCGC TGCCTGCTCC 120

CGCCACCCTA GGAGGCGCGG TGCCACCCAC TACTCTGTCC TCTGCCTGTG CTCCGTGCCC 180

GACCCTATCC CGGCGGAGTC TCCCCATCCT CCTTTGCTTT CCGACTGCCC AAGGCACTTT 240

CAATCTCAAT CTCTTCTCTC TCTCTCTCTC TCTCTCTGTC TCTCTCTCTC TCTCTCTCTC 300

TCTCTCTCTC GCAGGGTGGG GGGAAGAGGA GGAGGAATTC TTTCCCCGCC TAACATTTCA 360

AGGGACACAA TTCACTCCAA GTCTCTTCCC TTTCCAAGCC GCTTCCGAAG TGCTCCCGGT 420

GCCCGCAACT CCTGATCCCA ACCCGCGAGA GGAGCCTCTG CGACCTCAAA GCCTCTCTTC 480

CTTCTCCCTC GCTTCCCTCC TCCTCTTGCT ACCTCCACCT CCACCGCCAC CTCCACCTCC 540

GGCACCCACC CACCGCCGCC GCCGCCACCG GCAGCGCCTC CTCCTCTCCT CCTCCTCCTC 600

CCCTCTTCTC TTTTTGGCAG CCGCTGGACG TCCGGTGTTG ATGGTGGCAG CGGCGGCAGC 660

CTAAGCAACA GCAGCCCTCG CAGCCCGCCA GCTCGCGCTC GCCCCGCCGG CGTCCCCAGC 720

CCTATCACCT CATCTCCCGA AAGGTGCTGG GCAGCTCCGG GGCGGTCGAG GCGAAGCGGC 780

```
TGCAGCGGCG GTAGCGGCGG CGGGAGGCAG G ATG AGC GCA CGC GGT GAG GGC      832
                                   Met Ser Ala Arg Gly Glu Gly
                                     1               5

GCG GGG CAG CCG TCC ACT TCA GCC CAG GGA CAA CCT GCC GCC CCA GCG      880
Ala Gly Gln Pro Ser Thr Ser Ala Gln Gly Gln Pro Ala Ala Pro Ala
          10                  15                  20

CCT CAG AAG AGA GGA CGC GGC CGC CCC AGG AAG CAG CAG CAA GAA CCA      928
Pro Gln Lys Arg Gly Arg Gly Arg Pro Arg Lys Gln Gln Gln Glu Pro
     25                  30                  35

ACC GGT GAG CCC TCT CCT AAG AGA CCC AGG GGA AGA CCC AAA GGC AGC      976
Thr Gly Glu Pro Ser Pro Lys Arg Pro Arg Gly Arg Pro Lys Gly Ser
 40                  45                  50                  55

AAA AAC AAG AGT CCC TCT AAA GCA GCT CAA AAG AAA GCA GAA GCC ACT     1024
Lys Asn Lys Ser Pro Ser Lys Ala Ala Gln Lys Lys Ala Glu Ala Thr
                 60                  65                  70

GGA GAA AAA CGG CCA AGA GGC AGA CCT AGG AAA TGG CCA CAA CAA GTT     1072
Gly Glu Lys Arg Pro Arg Gly Arg Pro Arg Lys Trp Pro Gln Gln Val
             75                  80                  85

GTT CAG AAG AAG CCT GCT CAG GAG GAA ACT GAA GAG ACA TCC TCA CAA     1120
Val Gln Lys Lys Pro Ala Gln Glu Glu Thr Glu Glu Thr Ser Ser Gln
          90                  95                  100

GAG TCT GCC GAA GAG GAC TAGGGGGCGC AACGTTCGAT TTCTACCTCA            1168
Glu Ser Ala Glu Glu Asp
     105                 110

GCAGCAGTTG GATCTTTTGA AGGGAGAAGA CACTGCAGTG ACCACTTATT CTGTATTGCC   1228

ATGGTCTTTC CACTTTCATC TGGGGTGGGG TGGGGTGGGG TGGGGGAGGG GGGGGTGGGG   1288

TGGGGAGAAA TCACATAACC TTAAAAAGGA CTATATTAAT CACCTTCTTT GTAATCCCTT   1348

CACAGTCCCA GGTTTAGTGA AAAACTGCTG TAAACACAGG GGACACAGCT TAACAATGCA   1408

ACTTTTAATT ACTGTTTTCT TTTTTCTTAA CCTACTAATA GTTTGTTGAT CTGATAAGCA   1468

AGAGTGGGCG GGTGAGAAAA ACCGAATTGG GTTTAGTCAA TCACTGCACT GCATGCAAAC   1528

AAGAAACGTG TCACACTTGT GACGTCGGGC ATTCATATAG GAAGAACGCG GTGTGTAACA   1588

CTGTGTACAC CTCAAATACC ACCCCAACCC ACTCCCTGTA GTGAATCCTC TGTTTAGAAC   1648

ACCAAAGATA AGGACTAGAT ACTACTTTCT CTTTTTCGTA TAATCTTGTA GACACTTACT   1708

TGATGATTTT TAACTTTTTA TTTCTAAATG AGACGAAATG CTGATGTATC CTTTCATTCA   1768

GCTAACAAAC TAGAAAAGGT TATGTTCATT TTTCAAAAAG GGAAGTAAGC AAACAAATAT   1828

TGCCAACTCT TCTATTTATG GATATCACAC ATATCAGCAG GAGTAATAAA TTTACTCACA   1888

GCACTTGTTT TCAGGACAAC ACTTCATTTT CAGGAAATCT ACTTCCTACA GAGCCAAAAT   1948
```

GCCATTTAGC AATAAATAAC ACTTGTCAGC CTCAGAGCAT TTAAGGAAAC TAGACAAGTA 2008

AAATTATCCT CTTTGTAATT TAATGAAAAG GTACAACAGA ATAATGCATG ATGAACTCAC 2068

CTAATTATGA GGTGGGAGGA GCGAAATCTA AATTTCTTTT GCTATAGTTA TACATCAATT 2128

TAAAAAGCAA AAAAAAAAAG GGGGGGGCAA TCTCTCTCTG TGTCTTTCTC TCTCTCTCTC 2188

CCTCTCCCTC TCTCTTTTCA TGTGTATCAG TTTCCATGAA AGACCTGAAT ACCACTTACC 2248

TCAAATTAAG CATATGTGTT ACTTCAAGTA ATACGTTTTG ACATAAGATG GTTGACCAAG 2308

GTGCTTTTCT TCGGCTTGAG TTCACCATCT CTTCATTCAA ACTGCACTTT TAGCCAGAGA 2368

TGCAATATAT CCCCACTACT CAATACTACC TCTGAATGTT ACAACGAATT TACAGTCTAG 2428

TACTTATTAC ATGCTGCTAT ACACAAGCAA TGCAAGAAAA AAACTTACTG GGTAGGTGAT 2488

TCTAATCATC TGCAGTTCTT TTTGTACACT TAATTACAGT TAAAGAAGCA ATCTCCTTAC 2548

TGTGTTTCAG CATGACTATG TATTTTTCTA TGTTTTTTTA ATTAAAAATT TTTAAAATAC 2608

TTGTTTCAGC TTCTCTGCTA GATTTCTACA TTAACTTGAA AATTTTTTAA CCAAGTCGCT 2668

CCTAGGTTCT TAAGGATAAT TTTCCTCAAT CACACTACAC ATCACACAAG ATTTGACTGT 2728

AATATTTAAA TATTACCCTC CAAGTCTGTA CCTCAAATGA ATTCTTTAAG GAGATGGACT 2788

AATTGACTTG CAAAGACCTA CCTCCAGACT TCAAAAGGAA TGAACTTGTT ACTTGCAGCA 2848

TTCATTTGTT TTTTCAATGT TTGAAATAGT TCAAACTGCA GCTAACCCTA GTCAAAACTA 2908

TTTTTGTAAA AGACATTTGA TAGAAAGGAA CACGTTTTTA CATACTTTTG CAAAATAAGT 2968

AAATAATAAA TAAAATAAAG CCAACCTTCA AAGAACTTGA AGCTTTGTAG GTGAGATGCA 3028

ACAAGCCCTG CTTTTGCATA ATGCAATCAA AAATATGTGT TTTTAAGATT AGTTGAATAT 3088

AAGAAAATGC TTGACAAATA TTTTCATGTA TTTTACACAA ATGTGATTTT TGTAATATGT 3148

CTCAACCAGA TTTATTTTAA ACGCTTCTTA TGTAGAGTTT TTATGCCTTT CTCTCCTAGT 3208

GAGTGTGCTG ACTTTTTAAC ATGGTATTAT CAACTGGGCC AGGAGGTAGT TTCTCATGAC 3268

GGCTTTTGTC AGTATGGCTT TTAGTACTGA AGCCAAATGA AACTCAAAAC CATCTCTCTT 3328

CCAGCTGCTT CAGGGAGGTA GTTTCAAAGG CCACATACCT CTCTGAGACT GGCAGATCGC 3388

TCACTGTTGT GAATCACCAA AGGAGCTATG GAGAGAATTA AAACTCAACA TTACTGTTAA 3448

CTGTGCGTTA AATAAGCAAA TAAACAGTGG CTCATAAAAA TAAAAGTCGC ATTCCATATC 3508

TTTGGATGGG CCTTTTAGAA ACCTCATTGG CCAGCTCATA AAATGGAAGC AATTGCTCAT 3568

GTTGGCCAAA CATGGTGCAC CGAGTGATTT CCATCTCTGG TAAAGTTACA CTTTTATTTC 3628

CTGTATGTTG TACAATCAAA ACACACTACT ACCTCTTAAG TCCCAGTATA CCTCATTTTT 3688

```
CATACTGAAA AAAAAAGCTT GTGGCCAATG GAACAGTAAG AACATCATAA AATTTTTATA  3748
TATATAGTTT ATTTTTGTGG GAGATAAATT TTATAGGACT GTTCTTTGCT GTTGTTGGTC  3808
GCAGCTACAT AAGACTGGAC ATTTAACTTT TCTACCATTT CTGCAAGTTA GGTATGTTTG  3868
CAGGAGAAAA GTATCAAGAC GTTTAACTGC AGTTGACTTT CTCCCTGTTC CTTTGAGTGT  3928
CTTCTAACTT TATTCTTTGT TCTTTATGTA GAATTGCTGT CTATGATTGT ACTTTGAATC  3988
GCTTGCTTGT TGAAAATATT TCTCTAGTGT ATTATCACTG TCTGTTCTGC ACAATAAACA  4048
TAACAGCCTC TGTGATCCC                                               4067
```

Claims

1. Multi-tumor Aberrant Growth (MAG) gene having the nucleotide sequence of any one of the strands of any one of the members of the High Mobility Group protein genes or LIM protein genes, including modified versions thereof.

2. Multi-tumor Aberrant Growth (MAG) gene as claimed in claim 1 having essentially the nucleotide sequence of the HMGI-C gene as depicted in figure 7, or the complementary strand thereof, including modified or elongated versions of both strands.

3. Multi-tumor Aberrant Growth (MAG) gene as claimed in claim 1 having essentially the nucleotide sequence of the LPP gene as depicted in figure 5, or the complementary strand thereof, including modified or elongated versions of both strands.

4. Multi-tumor Aberrant Growth (MAG) gene as claimed in claim 1, 2 or 3 for use as a starting point for designing suitable expression-modulating compounds or techniques for the treatment of non-physiological proliferation phenomena in human or animal.

5. Multi-tumor Aberrant Growth (MAG) gene as claimed in claim 1, 2 or 3 for use as a starting point for designing suitable nucleotide probes for (clinically/medically) diagnosing cells having a non-physiological proliferative capacity as compared to wildtype cells.

6. Protein encoded by the Multi-tumor Aberrant Growth (MAG) gene as claimed in claim 1, 2 or 3 for use as a starting point for preparing suitable antibodies for (clinically/medically) diagnosing cells having a non-physiological proliferative capacity as compared to wildtype cells.

7. Derivatives of the MAG gene as claimed in claim 1, 2 or 3 for use in diagnosis and the preparation of therapeutical compositions, wherein the derivatives are selected from the group consisting of sense and anti-sense cDNA or fragments thereof, transcripts of the gene or practically usable fragments thereof, fragments of the gene or its complementary strand, proteins encoded by the gene or fragments thereof, antibodies directed to the gene, the cDNA, the transcript, the protein or the fragments thereof, as well as antibody fragments.

8. In situ diagnostic method for diagnosing cells having a non-physiological proliferative capacity, comprising at least some of the following steps:

   a) designing a set of nucleotide probes based on the information obtainable from the nucleotide sequence of the MAG gene as claimed in claim 1 or 2, wherein one of the probes is hybridisable to a region of the aberrant gene substantially mapping at the same locus as a corresponding region of the wildtype gene and the other probe is hybridisable to a region of the aberrant gene mapping at a different locus than a corresponding region of the wildtype gene;
   b) incubating one or more interphase or metaphase chromosomes or cells having a non-physiological proliferative capacity, with the probe under hybridising conditions; and
   c) visualising the hybridisation between the probe and th gene.

9. Method of diagnosing cells having a non-physiological proliferative capacity, comprising at least some of the following steps:

a) taking a biopsy of the cells to be diagnosed;
b) isolating a suitable MAG gene-related macromolecule therefrom;
c) analysing the macromolecule thus obtained by comparison with a wildtype reference molecule preferably from the same individual.

10. Method as claimed in claim 9, comprising at least some of the following steps:

a) taking a biopsy of the cells to be diagnosed;
b) extracting total RNA thereof;
c) preparing at least one first strand cDNA of the mRNA species in the total RNA extract, which cDNA comprises a suitable tail;
d) performing a PCR and/or RT-PCR using a MAG gene specific primer and a tail-specific and/or partner-specif/nested primer in order to amplify MAG gene specific cDNA's;
e) separating the PCR products on a gel to obtain a pattern of bands;
f) evaluating the presence of aberrant bands by comparison to wildtype bands, preferably originating from the same individual.

11. Method as claimed in claim 9, comprising at least some of the following steps:

a) taking a biopsy of the cells to be diagnosed;
b) isolating total protein therefrom;
c) separating the total protein on a gel to obtain essentially individual bands and optionally trnasferring the bands to a Western blot;
d) hybridising the bands thus obtained with antibodies directed against a part of the protein encoded by the remaining part of the MAG gene and against a part of the protein encoded by the substitution part of the MAG gene;
e) visualising the antigen-antibody reactions and establishing the presence of aberrant bands by comparison with bands from wildtype proteins, preferably originating from the same individual.

12. Method as claimed in claim 9, comprising at least some of the following steps:

a) taking a biopsy of the cells to be diagnosed;
b) isolating total DNA therefrom;
c) digesting the DNA with one or more so-called "rare cutter" restriction enzymes;
d) separating the digest thus prepared on a gel to obtain a separation pattern;
e) optionally transfering the separation pattern to a Southern blot;
f) hybridising the separation pattern in the gel or on the blot with one or more informative probes under hybridising conditions;
g) visualising the hybridisations and establishing the presence of aberrant bands by comparison to wildtype bands, preferably originating from the same individual.

13. Method as claimed in any one of the claims 8-12, wherein the cells having a non-physiological proliferative capacity are selected from the group consisting of the mesenchymal tumors hamartomas (e.g. breast and lung), lipomas, pleomorphic salivary gland adenomas, uterine leiomyomas, angiomyxomas, fibroadenomas of the breast, polyps of the endometrium, atherosclerotic plaques, and other benign tumors as well as various malignant tumors, including but not limited to sarcomas (e.g. rhabdomyosarcoma, osteosarcoma) and carcinomas (e.g. of breast, lung, skin, thyroid).

14. Anti-sense molecules of a MAG gene as claimed in claim 1, 2 or 3 for use in the treatment of diseases involving cells having a non-physiological proliferative capacity by modulating the expression of the gene.

15. Expression inhibitors of the MAG gene as claimed in claim 1, 2 or 3 for use in the treatment of diseases involving cells having a non-physiological proliferative capacity.

16. Diagnostic kit for performing the method as claimed in claim 8, comprising a suitable set of labeled nucleotide probes.

17. Diagnostic kit for performing the method as claimed in claim 10, comprising a suitable set of labeled probes.

18. Diagnostic kit for performing the method as claimed in claim 11, comprising a suitable set of lab led MAG gene specific and tail specific PCR primers.

19. Diagnostic kit for performing the method as claimed in claim 11, comprising a suitable set of labeled probes, and suitable rare cutting restriction enzymes.

20. Method for isolating other MAG genes based on the existence of a fusion gene, fusion transcript or fusion protein in a tumor cell by using at least a part of a MAG gene for designing molecular tools (probes, primers etc.).

21. MAG genes obtainable by the method of claim 20.

22. MAG genes as claimed in claim 21 for use in diagnostic or therapeutic methods.

Fig. 1-1

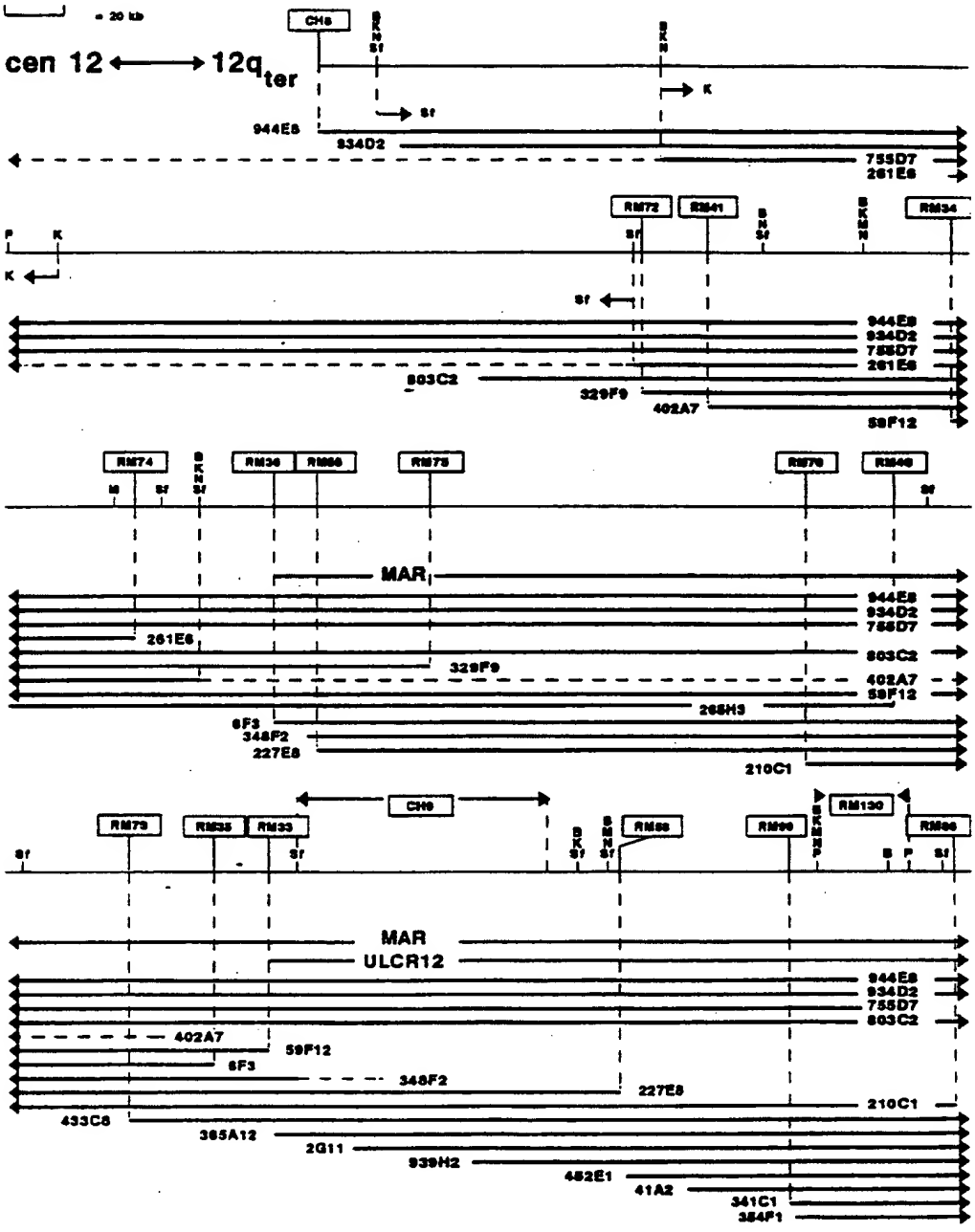

cen 12
12q ter
CH6
944E8
934D2
755D7
261E8
RM72
RM34
803C2
329F9
402A7
59F12
RM74
RM36
RM75
RM76
MAR
265H3
6F3
348F2
227E8
210C1
CH9
RM79
RM35
RM33
RM88
RM90
RM130
ULCR12
433C8
365A12
2G11
939H2
452E1
41A2
341C1
384F1

Fig. 1-2

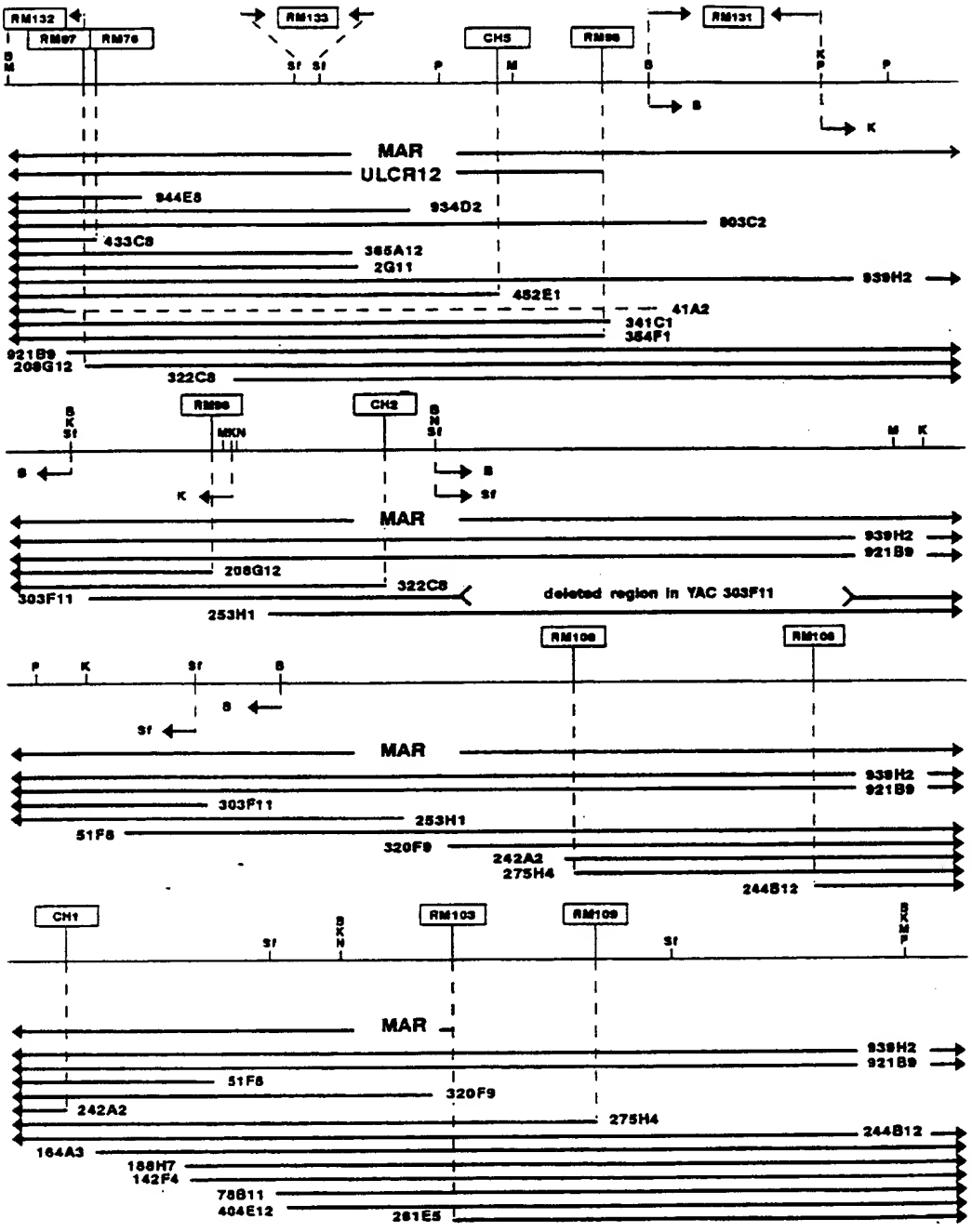

RM132
RM97
RM76
RM133
CH5
RM98
RM131
MAR
ULCR12
944E8
934D2
803C2
433C8
385A12
2G11
939H2
452E1
41A2
341C1
364F1
921B9
209G12
322C8
RM98
CH2
MAR
939H2
921B9
209G12
322C8
303F11
deleted region in YAC 303F11
253H1
RM108
RM106
MAR
939H2
921B9
303F11
253H1
51F8
320F9
242A2
275H4
244B12
CH1
RM103
RM109
MAR
939H2
921B9
51F8
320F9
242A2
275H4
244B12
164A3
188H7
142F4
78B11
404E12
261E5

Fig. 1-3

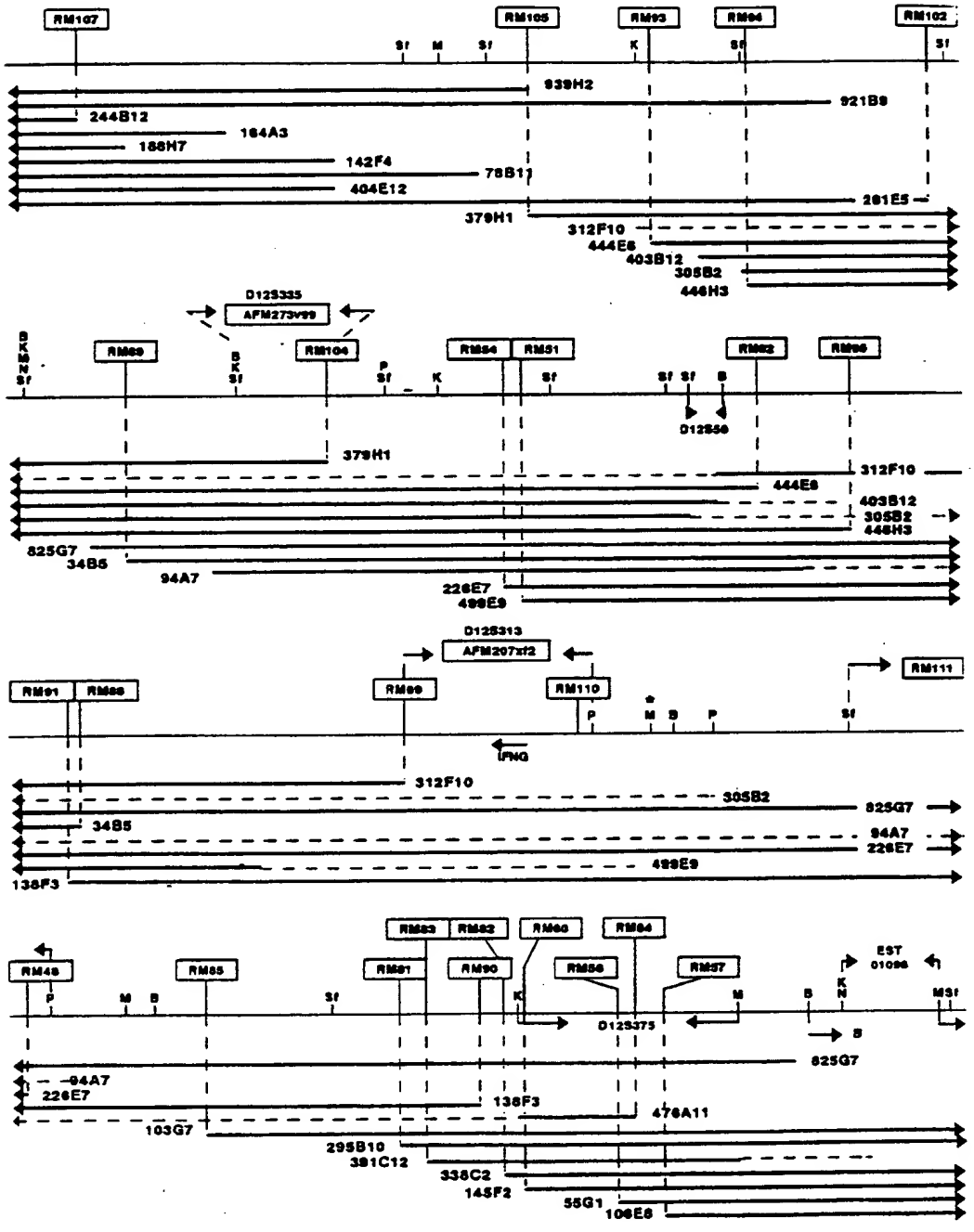
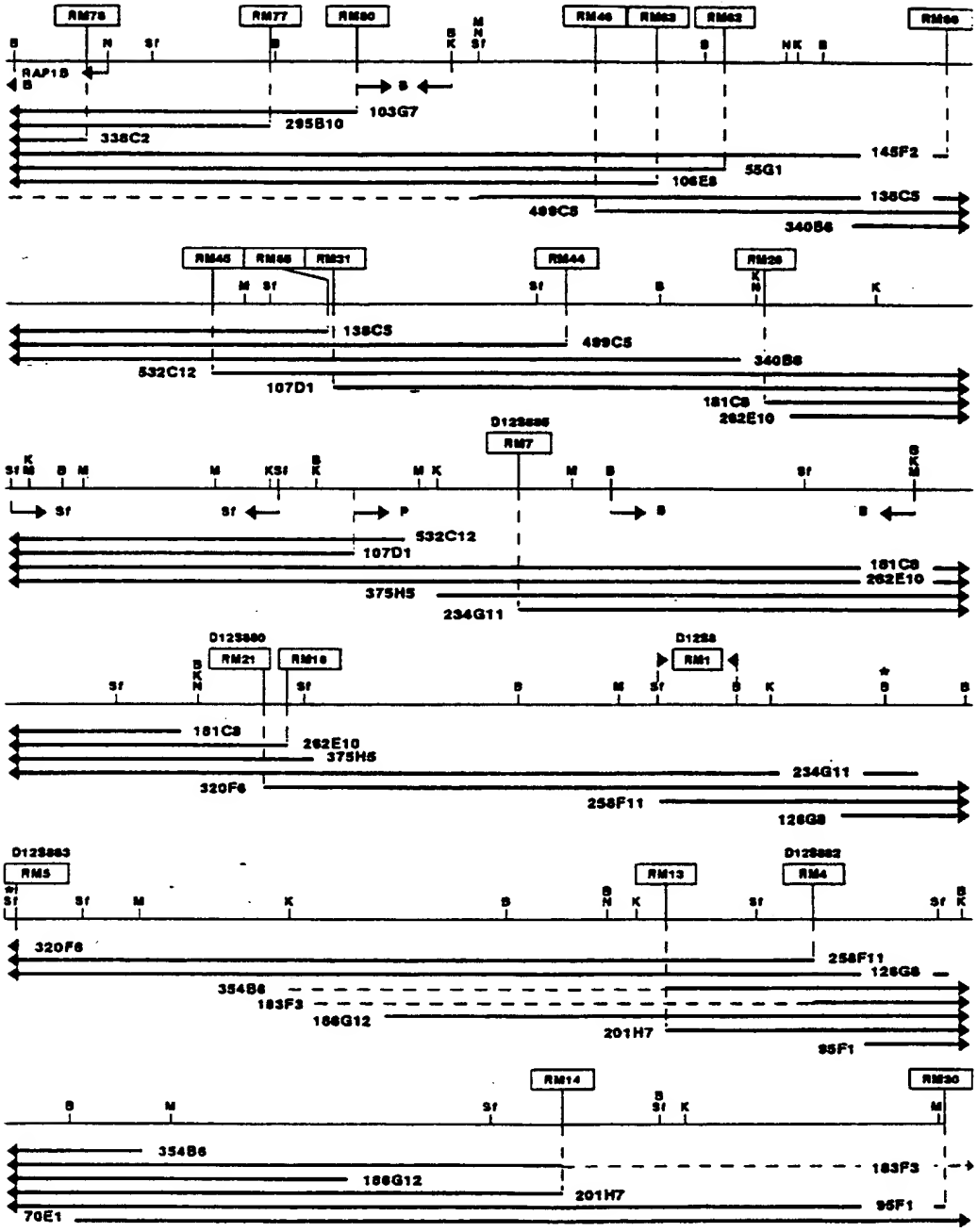

RM107
RM105
RM93
RM94
RM102
939H2
921B9
244B12
164A3
188H7
142F4
78B11
404E12
261E5
379H1
312F10
444E6
403B12
305B2
446H3
D12S335
AFM273vg9
RM89
RM104
RM84
RM81
RM82
RM96
D12S58
379H1
312F10
444E6
403B12
305B2
446H3
825G7
34B5
94A7
226E7
499E9
D12S313
AFM207xf2
RM91
RM88
RM99
RM110
RM111
PNG
312F10
305B2
825G7
34B5
94A7
226E7
499E9
138F3
RM48
RM85
RM83
RM82
RM90
RM86
RM84
RM87
EST
01096
D12S375
825G7
94A7
226E7
138F3
476A11
103G7
295B10
391C12
338C2
145F2
55G1
106E6

Fig. 1-4


RM75
RM77
RM80
RM46
RM63
RM62
RM64
RAP1B
103G7
295B10
338C2
145F2
55G1
106E8
138C5
499C8
340B6
RM45
RM66
RM31
RM44
RM29
138C5
499C8
340B6
532C12
107D1
181C8
262E10
D12S86
RM7
532C12
107D1
181C8
262E10
375H5
234G11
D12S80
RM21
RM16
D12S4
RM1
181C8
262E10
375H5
234G11
320F6
258F11
126G8
D12S83
RM5
RM13
D12S82
RM4
320F6
258F11
126G8
354B6
183F3
186G12
201H7
95F1
RM14
RM30
354B6
183F3
186G12
201H7
95F1
70E1

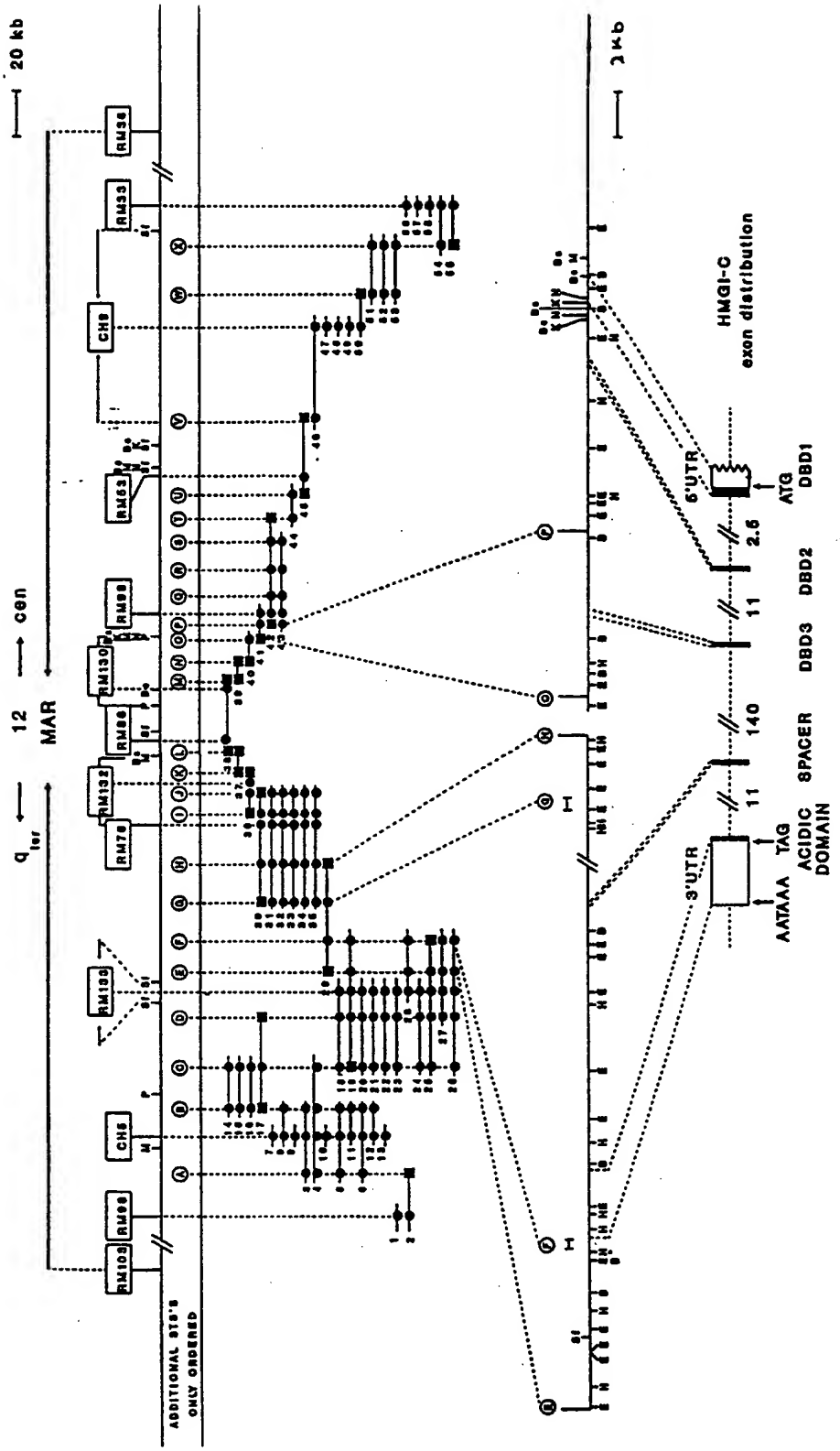

20 kb
2 kb
cen
12
MAR
qter
RM36
RM33
CH9
RM63
RM88
RM130
RM86
RM132
RM70
RM193
CH8
RM98
RM103
ADDITIONAL STS'S ONLY ORDERED
HMGI-C
exon distribution
5'UTR
ATG
DBD1
DBD2
DBD3
2.6
11
140
11
SPACER
ACIDIC DOMAIN
TAG
3'UTR
AATAAA

Fig. 2

Fig. 3

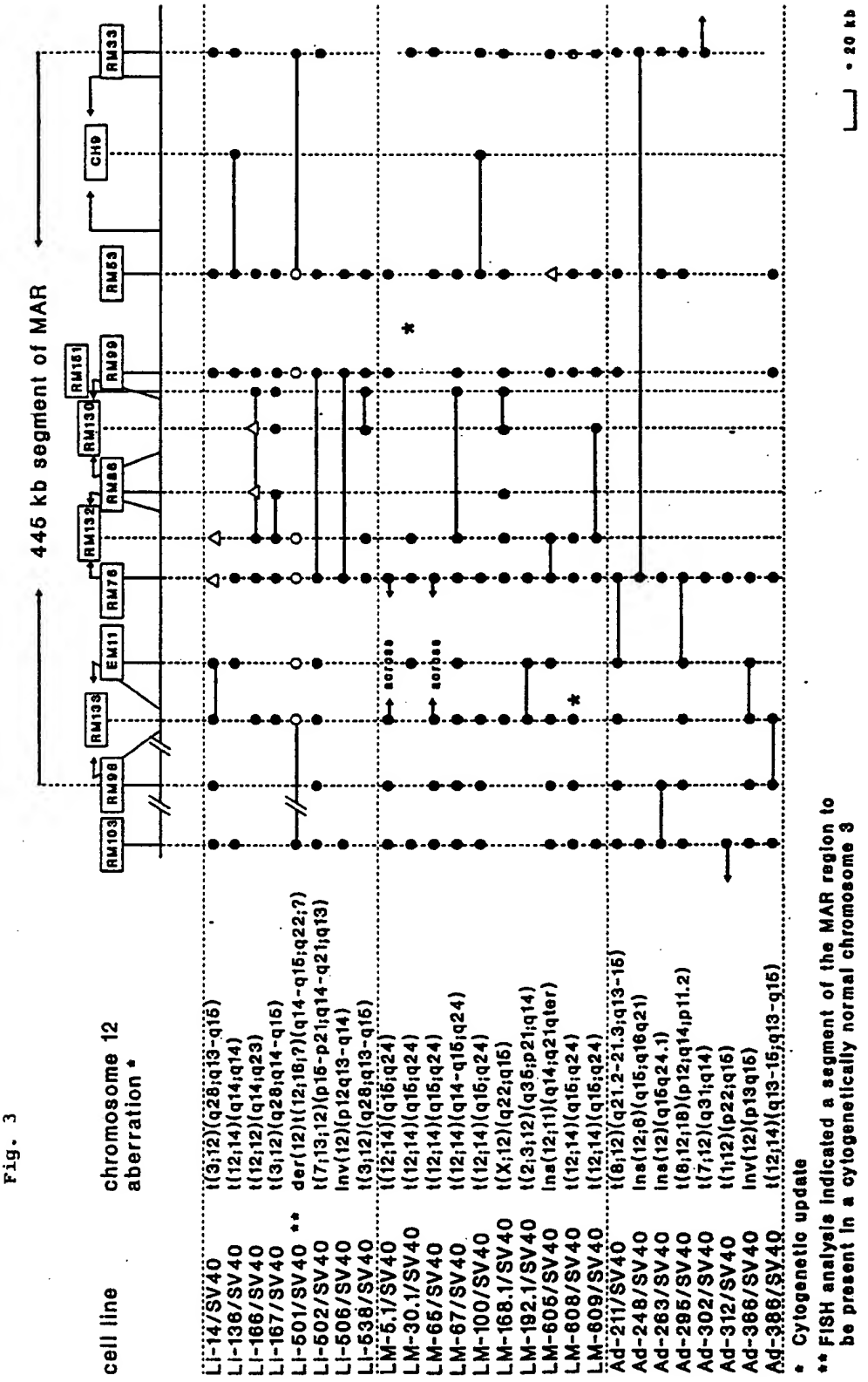
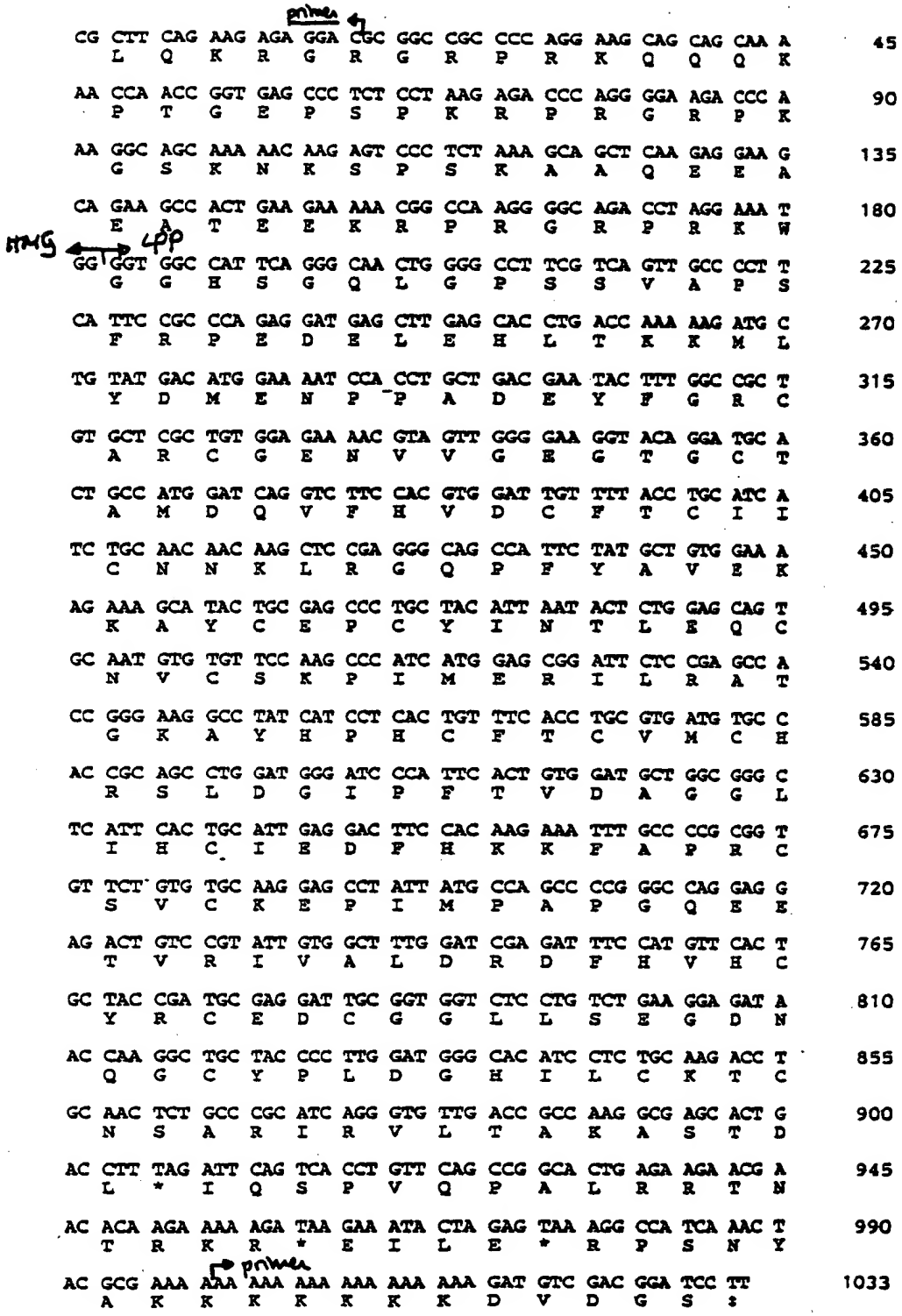

446 kb segment of MAR
RM103
RM98
RM133
EM11
RM76
RM132
RM86
RM130
RM151
RM99
RM53
CH9
RM33
cell line
chromosome 12 aberration *
LI-14/SV40 t(3;12)(q28;q13-q16)
LI-136/SV40 t(12;14)(q14;q14)
LI-166/SV40 t(12;12)(q14;q23)
LI-167/SV40 t(3;12)(q28;q14-q16)
LI-501/SV40 ** der(12)t(12;16;?)(q14-q15;q22;?)
LI-502/SV40 t(7;13;12)(p15-p21;q14-q21;q13)
LI-506/SV40 Inv(12)(p12q13-q14)
LI-538/SV40 t(3;12)(q28;q13-q15)
LM-5.1/SV40 t(12;14)(q15;q24)
LM-30.1/SV40 t(12;14)(q15;q24)
LM-65/SV40 t(12;14)(q15;q24)
LM-67/SV40 t(12;14)(q14-q15;q24)
LM-100/SV40 t(12;14)(q15;q24)
LM-168.1/SV40 t(X;12)(q22;q15)
LM-192.1/SV40 t(2;3;12)(q35;p21;q14)
LM-605/SV40 Ins(12;11)(q14;q21qter)
LM-608/SV40 t(12;14)(q15;q24)
LM-609/SV40 t(12;14)(q15;q24)
Ad-211/SV40 t(8;12)(q21.2-21.3;q13-16)
Ad-248/SV40 Ins(12;8)(q15;q16q21)
Ad-263/SV40 Ins(12)(q15q24.1)
Ad-295/SV40 t(8;12;18)(p12;q14;p11.2)
Ad-302/SV40 t(7;12)(q31;q14)
Ad-312/SV40 t(1;12)(p22;q15)
Ad-366/SV40 Inv(12)(p13q15)
Ad-386/SV40 t(12;14)(q13-15;q13-q15)
sarcoma
sarcoma
* Cytogenetic update
** FISH analysis indicated a segment of the MAR region to be present in a cytogenetically normal chromosome 3
20 kb

Fig. 4



CG CTT CAG AAG AGA GGA CGC GGC CGC CCC AGG AAG CAG CAG CAA A 45
L Q K R G R G R P R K Q Q Q K

AA CCA ACC GGT GAG CCC TCT CCT AAG AGA CCC AGG GGA AGA CCC A 90
P T G E P S P K R P R G R P K

AA GGC AGC AAA AAC AAG AGT CCC TCT AAA GCA GCT CAA GAG GAA G 135
G S K N K S P S K A A Q E E A

CA GAA GCC ACT GAA GAA AAA CGG CCA AGG GGC AGA CCT AGG AAA T 180
E A T E E K R P R G R P R K W

GG GGT GGC CAT TCA GGG CAA CTG GGG CCT TCG TCA GTT GCC CCT T 225
G G H S G Q L G P S S V A P S

CA TTC CGC CCA GAG GAT GAG CTT GAG CAC CTG ACC AAA AAG ATG C 270
F R P E D E L E H L T K K M L

TG TAT GAC ATG GAA AAT CCA CCT GCT GAC GAA TAC TTT GGC CGC T 315
Y D M E N P P A D E Y F G R C

GT GCT CGC TGT GGA GAA AAC GTA GTT GGG GAA GGT ACA GGA TGC A 360
A R C G E N V V G E G T G C T

CT GCC ATG GAT CAG GTC TTC CAC GTG GAT TGT TTT ACC TGC ATC A 405
A M D Q V F H V D C F T C I I

TC TGC AAC AAC AAG CTC CGA GGG CAG CCA TTC TAT GCT GTG GAA A 450
C N N K L R G Q P F Y A V E K

AG AAA GCA TAC TGC GAG CCC TGC TAC ATT AAT ACT CTG GAG CAG T 495
K A Y C E P C Y I N T L E Q C

GC AAT GTG TGT TCC AAG CCC ATC ATG GAG CGG ATT CTC CGA GCC A 540
N V C S K P I M E R I L R A T

CC GGG AAG GCC TAT CAT CCT CAC TGT TTC ACC TGC GTG ATG TGC C 585
G K A Y H P H C F T C V M C H

AC CGC AGC CTG GAT GGG ATC CCA TTC ACT GTG GAT GCT GGC GGG C 630
R S L D G I P F T V D A G G L

TC ATT CAC TGC ATT GAG GAC TTC CAC AAG AAA TTT GCC CCG CGG T 675
I H C I E D F H K K F A P R C

GT TCT GTG TGC AAG GAG CCT ATT ATG CCA GCC CCG GGC CAG GAG G 720
S V C K E P I M P A P G Q E E

AG ACT GTC CGT ATT GTG GCT TTG GAT CGA GAT TTC CAT GTT CAC T 765
T V R I V A L D R D F H V H C

GC TAC CGA TGC GAG GAT TGC GGT GGT CTC CTG TCT GAA GGA GAT A 810
Y R C E D C G G L L S E G D N

AC CAA GGC TGC TAC CCC TTG GAT GGG CAC ATC CTC TGC AAG ACC T 855
Q G C Y P L D G H I L C K T C

GC AAC TCT GCC CGC ATC AGG GTG TTG ACC GCC AAG GCG AGC ACT G 900
N S A R I R V L T A K A S T D

AC CTT TAG ATT CAG TCA CCT GTT CAG CCG GCA CTG AGA AGA ACG A 945
L * I Q S P V Q P A L R R T N

AC ACA AGA AAA AGA TAA GAA ATA CTA GAG TAA AGG CCA TCA AAC T 990
T R K R * E I L E * R P S N Y

AC GCG AAA AAA AAA AAA AAA AAA AAA GAT GTC GAC GGA TCC TT 1033
A K K K K K K K D V D G S :

Fig. 5-1

"GEN3" -> partial cDNA sequence

```
GTCACTTTTA TTTGGGGGTG TGGACAGCTG CTTTCCCAGG GGAGTACTTC TTACAGTGGG    60
ATTTCAAGAC AAGATCGGCC TGAAGAAAAA TTATATTTGT ATATTTTTTA AAAAGTGGGA   120
ACTTTGAGGC TCAGAGACAG AGCAGAAGAC AGAACCTGGT CTTCTGATTC CCTGTGTTCT   180
GCTTTTTTCA TTGTTCCACT GGACGCTCAT CAGAGGGAAG ATCTTTTTCC TCAATTGATT   240
CCAACAATGT CTCACCCATC TTGGCTGCCA CCCAAAAGCA CTGGTGAGCC CCTCGGCCAT   300
GTGCCTGCAC GGATGGAGAC CACCCATTCC TTTGGGAACC CCAGCATTTC AGTGTCTACA   360
CAACAGCCAC CCAAAAAGTT TGCCCCGGTA GTTGCTCCAA AACCTAAGTA CAACCCATAC   420
AAACAACCTG GAGGTGAGGG TGATTTTCTT CCACCCCCAC CTCCACCTCT AGATGATTCC   480
AGTGCCCTTC CATCTATCTC TGGAAACTTT CCTCCTCCAC CACCTCTTGA TGAAGAGGCT   540
TTCAAAGTAC AGGGGAATCC CGGAGGCAAG ACACTTGAGG AGAGGCGCTC CAGCCTGGAC   600
GCTGAGATTG ACTCCTTGAC CAGCATCTTG GCTGACCTTG AGTGCAGCTC CCCCTATAAG   660
CCTCGGCCTC CACAGAGCTC CACTGGTTCA ACAGCCTCTC CTCCAGTTTC GACCCCAGTC   720
ACAGGACACA AGAGAATGGT CATCCCGAAC CAACCCCCTC TAACAGCAAC CAAGAAGTCT   780
ACATTGAAAC CACAGCCTGC ACCCCAGGCT GGACCCATCC CTGTGGCTCC AATCGGAACA   840
CTCAAACCCC AGCCTCAGCC AGTCCCAGCC TCCTACACCA CGGCCTCCAC TTCTTCAAGG   900
CCTACCTTTA ATGTGCAGGT GAAGTCAGCC CAGCCCAGCC CTCATTATAT GGCTGCCCCT   960
TCATCAGGAC AAATTTATGG CTCAGGGCCC CAGGGCTATA ACACTCAGCC AGTTCCTGTC  1020
TCTGGGCAGT GTCCACCTCC TTCAACACGG GGAGGCATGG ATTATGCCTA CATTCCACCA  1080
CCAGGACTTC AGCCGGAGCC TGGGTATGGG TATGCCCCCA ACCAGGGACG CTATTATGAA  1140
GGCTACTATG CAGCAGGGCC AGGCTATGGG GGCAGAAATG ACTCTGACCC TACCTATGGT  1200
CAACAAGGTC ACCCAAATAC CTGGAAACGG GAACCAGGGT ACACTCCTCC TGGAGCAGGG  1260
AACCAGAACC CTCCTGGGAT GTATCCAGTC ACTGGTCCCA AGAAGACCTA TATCACAGAT  1320
CCTGTTTCAG CCCCCTGTGC GCCACCATTG CAGCCAAAGG GTGGCCATTC AGGGCAACTG  1380
GGGCCTTCGT CAGTTGCCCC TTCATTCCGC CCAGAGGATG AGCTTGAGCA CCTGACCAAA  1440
AAGATGCTGT ATGACATGGA AAATCCACCT GCTGACGAAT ACTTTGGCGG CTGTGCTCGC  1500
TGTCGAGAAA ACGTAGTTGG GGAAGGTACA GGATGCACTG CCATGGATCA GGTCTTCCAC  1560
GTGGATTGTT TTACCTGCAT CATCTGCAAC AACAAGCTCC GAGGGCAGCC ATTCTATGCT  1620
```

Fig. 5-2

```
GTGGAAAAGA AAGCATACTG CGAGCCCTGC TACATTAATA CTCTGGAGCA GTGCAATGTG  1680
TGTTCCAAGC CCATCATGGA GCGGATTCTC CGAGCCACCG GGAAGGCCTA TCATCCTCAC  1740
TGTTTCACCT GCGTGATGTG CCACCGCAGC CTGGATGGGA TCCCATTCAC TGTGGATGCT  1800
GGCGGGCTCA TTCACTGCAT TGAGGACTTC CACAAGAAAT TTGCCCCGCG GTGTTCTGTG  1860
TGCAAGGAGC CTATTATGCC AGCCCCGGGC CAGGAGGAGA CTGTCCGTAT TGTGGCTTTG  1920
GATCGAGATT TCCATGTTCA CTGCTACCGA TGCGAGGATT GCGGTGGTCT CCTGTCTGAA  1980
GGAGATAACC AAGGCTGCTA CCCCTTGGAT GGGCACATCC TCTGCAAGAC CTGCAACTCT  2040
GCCCGCATCA GGGTGTTGAC CGCCAAGGCG AGCACTGACC TTTAGATTCA GTCACCTGTT  2100
CAGCCGGCAC TGAGAAGAAC GAACACAAGA AAAAGATAAG AAATACTAGA GTAAAGGCCA  2160
TCAAACTACG CGATAGTCAC TGTTCTTCAT CTGCTATTAA CCTTGCCTTA GAAACACATA  2220
AATTATGAGA TTTTTTTTTA AAAGTTGTTA CCAAATACAC ATTTCACATT GAATCATGTA  2280
GGATCTTGAT GGGCCTTTGT TCCCAAGGAC TTCCACATTT TTGCACAGAT TATGCTCCAT  2340
CCCTTCACTT CTGCATTCCT GTAACTTTTA ATCCCTATGT TTGTCTCACT TTTCATCTGG  2400
TTGAATGGCT TTTCTTAGTG TGGTATTTGC TGTCACATAG TTTTTTCCTG GGTGAGTCTG  2460
CCAACTCACA GGTGCTTTTA GGCTTGAAAT CTCCATCCTA TCATTTCCGT TTTGCCTGTG  2520
ACTGTAAAGA GTAGCCATTC TTTTCCCATG TATTGAAGAG GATATTCTTC TCTTGCTTTA  2580
TACTACTCAC GTCCTTGGGG AGGGAAATGC ACAATTTTTT TTTGTTAGGC TGTAAAGAAT  2640
TTAAGCTGTA AATTACATAA GTTAGAACAA GCCCAAATTT AATTTGCAAC CATCAGAATT  2700
CAGAATCTAT AGTGACCAGT GATCAAGGCT AATTGGAAAA GAGTTATCGG CCCATAGCTA  2760
ATAAGTAGTG ACAGACAACC AAGCTTCAAT ATTTTTCTAA AGAAATTACA GGTGGGATAT  2820
GCTAGAAAAG GCATTTTGGG GTTATGTTTA AAAAAACATT ATTGTCCCAC AATATTACCT  2880
TAAGATTTTT CTTTTCCGCA CTACCTGAAC ATTGTAATAC AGACAAACTT GATTTCTTCT  2940
AGAAGATAAC ATTTTCAATA CTGTCCCACT TCTCATCTTA AAAATATTGT CATGTTTATT  3000
CTAATATCCA ACGCAACTAT CAAAATTGCC TTTTTCTCTA GAGGATGAAG GCTGTGAAAA  3060
AACCGTTCAA ATTCTCTTCT TTTTCTTTTT TATTACCAGG TCCATTTTGC CTGACAATTG  3120
CAAATCAGAG CATACAAAAT AAAACTGTGC AGTTTTGTTT GGTTTACTTT CAAAAGAGTA  3180
GAAAGCTTGA AAAGATTCTG AAACCACAGT TTCATTATTC TCATAATCCT TCTGCAACTG  3240
AAATTACATA TTGCAGGAGA CATTTTCATA TCATCAATGT GACATTTACA CCACACTTTC  3300
AAAGACAATC ACTGAAACAA AAATTGTCTT TATGAGCTAA AAATATGCAG AATCTCTGCC  3360
```

Fig. 5-3

```
TAGAATCTTT ATTCAAACTT TTATTAGCCA GTGAAACACT TGCTTGCCAA CTGCCAAGCC  3420
ATACTTATTA AGTTCGAACA TGTTTCACTT AAGGAGAGAC ACCTAGCTTA GTCATGGCAA  3480
GTTGCCATTT TGTAAACTAA GGATTTTGGA CTGAGATTTC TTAAATCTTT CTTCAAATCT  3540
CCCACAAGTA TATACTTTTA AATTATGGAG TATTTTAAGT CTACAAAAAG GTATAAATAA  3600
TAATATAATG AATTCCTATA TACCTAATAC CCAGTTTAAG ACACCAAATA TAACAAGTAT  3660
AATTACATCC TCCAATGTAC CGTTTCCTTA TTCCACAGAT ATCTTTTTCA TTATTGTGAA  3720
GTGATGTTCA GATTTCTAGT TTTTTTTTCT AGTTTTTAAT TTTAACATCA GAACTGAAAT  3780
AAAAAATTAT GGATACGTGT TTTGAATTGC AAACTATTCC TCAGGAATTC CAATTAAATT  3840
TATTTTACTT GAATAGGAAT GATCATAAAA GTGATTCTTT TTTTGTGACT AGAAATTCTT  3900
AAGCCGATGG TCACTATAGC TCATCCTTAA TGTATGGCTC ATTTGCTTTT GTCACTAAAC  3960
GGTTTTGTGT TAGAACCACC AAAATTATAG CTTTTAAGAG CTTCCTTTGA CCACTGTCTT  4020
TTTCTTACCC TACTTCTCTT ATCTTTGATC GTATATTTCT CATAATGTGA AATATGATGA  4080
GATTCACTTA GGGGCAGCAT GTTAGTTTTG GGAGGCAATG TCAACTGTGT CTCTGAATTC  4140
CTGTCTTCCA AATTGAAGCC AGACCATGCT GATGACCTCA AGTAGCACTG ACTATTTGAC  4200
AATAGGGCTG ATAATGTAAT CGGCTTGAAT TTTGACTTAG TAACTTTTTA TGTAATACTT  4260
TCGGAGAAAT TCTCTTTAGG ACAAAGCAGA GAGTCCAATT TATTGAGGGA TAGATTGTAT  4320
CTC                                                                4323
```

Fig. 6

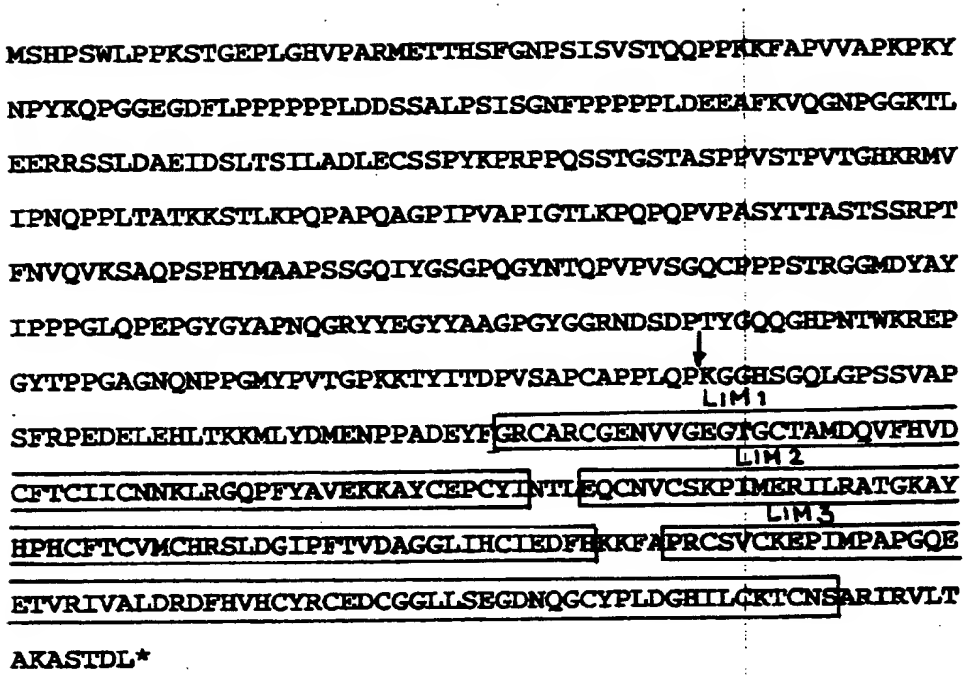

MSHPSWLPPKSTGEPLGHVPARMETTHSFGNPSISVSTQQPPKKFAPVVAPKPKY
NPYKQPGGEGDFLPPPPPPLDDSSALPSISGNFPPPPPLDEEAFKVQGNPGGKTL
EERRSSLDAEIDSLTSILADLECSSPYKPRPPQSSTGSTASPPVSTPVTGHKRMV
IPNQPPLTATKKSTLKPQPAPQAGPIPVAPIGTLKPQPQPVPASYTTASTSSRPT
FNVQVKSAQPSPHYMAAPSSGQIYGSGPQGYNTQPVPVSGQCPPPSTRGGMDYAY
IPPPGLQPEPGYGYAPNQGRYYEGYYAAGPGYGGRNDSDPTYGQQGHPNTWKREP
GYTPPGAGNQNPPGMYPVTGPKKTYITDPVSAPCAPPLQPKGGHSGQLGPSSVAP
LIM 1
SFRPEDELEHLTKKMLYDMENPPADEYFGRCARCGENVVGEGTGCTAMDQVFHVD
LIM 2
CFTCIICNNKLRGQPFYAVEKKAYCEPCYINTLEQCNVCSKPIMERILRATGKAY
LIM 3
HPHCFTCVMCHRSLDGIPFTVDAGGLIHCIEDFEKKFAPRCSVCKEPIMPAPGQE
ETVRIVALDRDFHVHCYRCEDCGGLLSEGDNQGCYPLDGHILCKTCNSARIRVLT
AKASTDL*

Fig. 7-1

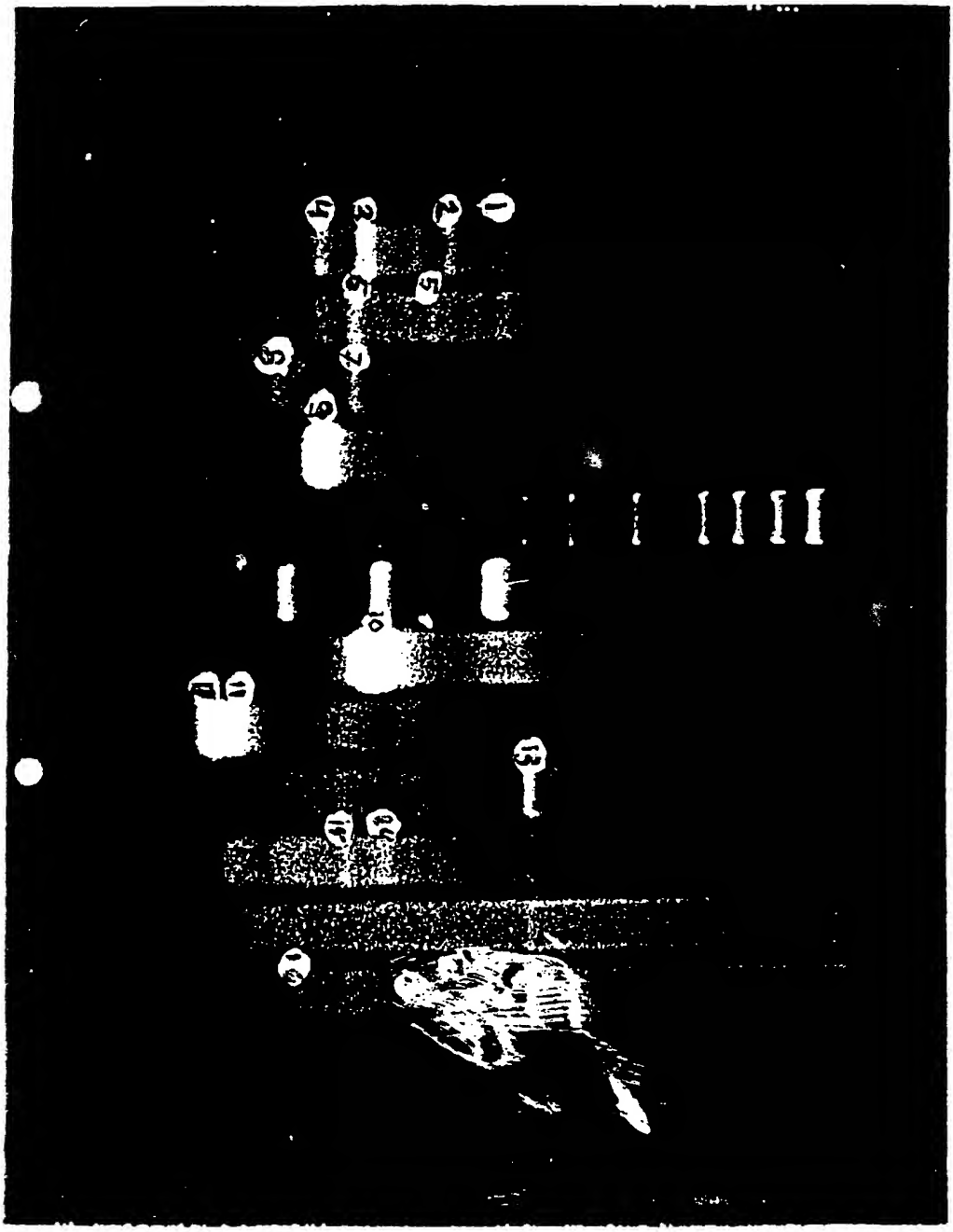
BASE COUNT 1119 a 988 c 791 g 1169 t
ORIGIN

```
   1 cttgaatctt ggggcaggaa ctcagaaaac ttccagccag ggcagcgcgc gcttggtgca
  61 agactcaggg gctagcagcc cgtcccccgc cgactctccg gtgccgccgc tgcctgctcc
 121 cgccaccccg ggagggcggg tgccacccac cactctgtcc tctgcctgtg ctccgtgccc
 181 gaccctatcc cggcggagtc tcccccatcc catttgcttt ccgactgccc aaggcaattt
 241 caatctcaat ctcttctctc tcictctctc tctctctgtc tctctctctc tctctctctc
 301 tctctctctc gcagggtggg gggaagaggg ggaggaattc tttccccgcc taacatttca
 361 agggaccacg ttcactccaa gtctcttccc tttcaaagcc gcttccgaag tgctccgggt
 421 gccccgcacc cctgatcccc accccggaga ggagcctctg cgacctcaaa gcctctcttc
 481 cttctccctc gcttccctcc tcctcttgct acctccacct ccaccgccac ctccacctcc
 541 ggcaccccac caccgccgcc gccgccaccg gcagcgcctc ctcctctcct cctcatcctc
 601 ccctcttctc ttttcggcag ccgctggacg tccggtgttg atggtggcag cggcggcagc
 661 ctaagcaaca gcagccctcg cagccccgcca gctcgcgctc gccccgcccg cgtccccagc
 721 cctatcacct catctcccga aaggtgctgg gcagctccgg ggcggtcgag gcgaagcggc
 781 tgcagcggcg gtagcggcgg cgggaggcag gatgagcgca cgcggtgagg gcgcgggcca
 841 gccgtccact tcagcccagg gacaacctgc cgccccagcg cctcagaaga gaggacgcgg
 901 ccgccccagc aagcagcagc aagaaccaac cggtgagccc tctcctaaga gacccagggg
 961 aagacccaaa ggcagcaaaa acaagagtcc gtctaaagca gctcaaaaga aagcagaagc
1021 cactggagaa aaacggccaa gaggcagacc taggaaatgg ccacaacaag ttgttcagaa
1081 gaagcctgct caggaggaaa ctgaagagac atcctcacaa gagtctgccg aagaggacta
1141 ggggggcgca cgttcgattt ctacctcagc agcagttgga tcttttgaag ggagaagaca
1201 ctgcagtgac cacttattct gtattgccat ggtctttcca ctttcatctg gggtggggtg
1261 gggtggggtg ggggaugggg gggtgggggtg gggagaaatc acataacctt aaaaaggact
1321 atattaatca ccttctcttgt aatcccttca cagtcccagg tttagtgaaa aactgctgta
1381 aacacagggg acacagctta acgatgcaac ttttaattac tgttttcttt tttcttaacc
1441 tactaatagt ttgttgatct gataagcaag agtgggcggg tgagaaaaac cgaattgggt
1501 ttagtcaatc actgcactgc atgcaaacaa gaaacgtgtc acacttgtga cgtcgggcat
1561 tcatatagga agaacgcggt gtgtaacact gtgtacacct caaataccac cccacccac
1621 tcccctgtagt gaatcctctg tttagaacac caaagataag gactagatac tactttctct
1681 ttttagtata atcttgtaga cacttacttg atgattttta actttttatt tctaaatgag
1741 acgaaatgct gatgtatcct ttcattcagc taacaaacta gaaaaggtta tgttcatttt
1801 tcaaaaaggg aagtaagcaa aacaaatatg ccaactcttc tatttatgga tatcacacat
1861 atcagccagga gtaataaatt tactcacagc acttgttttc aggacaacac ttcatttcca
1921 ggcaatctac ttcctacaga gccaaaatgc catttagcaa taaataacac ttgtcagcct
1981 cagagccatt aaggaaacta gacaagtaaa attatcctct ttgtaattta atgaaaaggt
2041 acaacagaat aatgcatgat gaactcacct aattatgagg tgggaggagc gaaatctaaa
2101 tttcttttgc tatagttata catcaattta aaaagcaaaa aaaaaaaggg gggggcaatc
2161 tntctctgtg tctttctctc tctctctccc tctccctctc tcttttcatg tgtatcagtt
2221 tcaatgaaag acctgaatac cacttaactc aaattaagca tatgtgttac ttcaagtaat
2281 acgttttgac ataagatggt tgaccaaggt gcttttctta ggcttgagtt caccatctct
2341 tcattcaaac tgcactttta gccagagatg caatatatcc ccactactca atactacctc
2401 tgaatgttac aacgaattta cagtctagta cttattacat gctgctatac acaagcaatg
2461 caagaaaaaa acttactggg taggtgattc taatcatctg cagttctttt tgtacactta
2521 attacagtta aagaagcaat ctccttactg tgtttcagca tgactatgta ttttctatg
2581 ttttttttaat taaaaatttt taaaatactt gtttcagctt ctctgctaga tttctacatt
2641 aacttgaaaa ttttttaacc aagtcgctcc taggttctta aggataattt tcctcaatca
2701 cactacacat cacacaagat ttgactgtaa tatttaaata ttaccctcca agtctgtacc
2761 tcaaatgaat tctttaagga ygtggactaa ttgacttgca aagcctacc tccagactta
2821 aaaaggaatg aacttgttac ttgcagcatt catttgtttt ttcaatgttt gaaatagttc
2881 aaactgcagc taacctagt caaaactatt tttgtaaaag acatttgata gaaaggaaca
2941 cgttttttaca tacttttgca aaataagtaa atattaaata aaataaagcc aaccttcaaa
3001 gaacttgaag ctttgtaggt gagatgcaac aagccctgct tttgcataat gcaatcaaaa
3061 atatgtgttt ttaagattag ttgaatataa gaaaatgctt gacaaatatt ttcatgtatt
3121 ttacacaaat gtgatttttg taatatgtct caaccagatt tattttaaac gcttcttatg
3181 tagagttttt atgcctttct ctcctagtga gtgtgctgac tttttaacat ggtattatca
3241 actgggccag gaggtagttt atcatgacgg cttttgtcag tatggctttt agtactgaag
3301 ccaaatgaaa ctcaaaacca tctatcttcc agctgcttca gggaggtagt ttcaaaggcc
3361 acatacctct ctgagactgg cagatcgctc actgttgtga atcaccaaag gagctatgga
3421 gagaattaaa actcaagatt actgttaact gtgcgttaaa taagcaaata aacagtggct
3481 cataaaaata aaagtcggat tccatatctt tggatgggcc ttttagaaac ctcattggcc
3541 agctcataaa atggaaycaa ttg tcatgt tggccaaaca tggtgcaccg agtgatttcc
3601 atctctggta aagttacact tttattt ct gtatgttgta caatcaaaac acactactac
3661 ctcttaagtc ccagtatacc tcattittca ta tgaaaaa aaaagcttgt ggccaatgga
```

Fig. 7-2

```
3721 acagtaagaa catcatanaa ttttatata tatagtttat ttttgtggga gataaatttt
3781 ataggactgt tatttgctgt tgttggtcgc agctacataa gactggacat ttaactttta
3841 taccatttct gcaagttayy tatgtttgca ggagaaaagt atcaagacgt ttaactgcag
3901 ttgacttttt ccctgttcct ttgagtgtct tctaacttta ttctttgttc tttatgtaga
3961 attgctgtct atgattgtac tttgaatcgc ttgcttgttg aaaatattto tctagtgtat
4021 tatcactgtc tgttctgcac aataaacata acagcctctg tgatccc
```

Fig. 8



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 20 1951

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	CHEMICAL ABSTRACTS, vol. 122, no. 7, 13 February 1995 Columbus, Ohio, US; abstract no. 73586t, SCHOENMAKERS,ERIC ET AL. 'Identification,molecular cloning and characterization of the chromosome 12 breakpoint cluster region of uterine leiomyomas.' page 267; * abstract * & GENES,CHROMOSOMES CANCER , vol. 11, no. 2, 1994 pages 106-118, ---	1,2,4-7	C12N15/12 C12N15/11 C07K14/47 C12Q1/68 C07K19/00 C07K16/18 A61K38/17
X	NUCLEIC ACIDS RESEARCH, vol. 19, no. 24, 1991 OXFORD GB, pages 6793-6797, G.MANFIOLETTI ET AL. 'cDNA cloning of the HMGI-C phosphoprotein ,a nuclear protein associated with neoplastic and undifferentiated phenotypes.' * the whole document * ---	1,2,4-7	
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 201, no. 1, 30 May 1994 ORLANDO, FL US, pages 63-70, UMESH A. PATEL ET AL. 'Expression and cDNA cloning of human HMGI-C phosphoprotein.' * the whole document * --- -/--	1,2,4-7	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
C07K
C12Q

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12 March 1996	Delanghe, L

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 20 1951

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	DATABASE WPI Section Ch, Week 8735 Derwent Publications Ltd., London, GB; Class B04, AN 87-245773 & JP-A-62 166 897 (TOYO SODA MFG KK) , 23 July 1987 * abstract * ---	6	
P,X	NUCLEIC ACIDS RESEARCH, vol. 23, no. 21, 1995 OXFORD GB, pages 4262-4266, KAI-YIN CHAU ET AL. 'The gene for the human architectural transcription factor HMGI-C consists of five exons each coding for a distinct functional element.' * the whole document * ---	1,2,4-7	
P,X	CHEMICAL ABSTRACTS, vol. 123, no. 1, 16 January 1995 Columbus, Ohio, US; abstract no. 6842f, VAN DE VEN,WIM J.M. ET AL. 'Molecular characterization of MAR,a multiple aberration region on human chromosome segment 12q13-q15 implicated in various solid tumors.' page 721; * abstract * & GENES,CHROMOSOMES CANCER, vol. 12, no. 4, 1995 pages 296-303, --- -/--	1,2,4-7	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12 March 1996	Delanghe, L

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 20 1951

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
T	CHEMICAL ABSTRACTS, vol. 123, no. 15, 9 October 1995 Columbus, Ohio, US; abstract no. 195315v, SCHOENMAKERS,ERIC ET AL. 'Recurrent rearrangements in the high mobility group protein gene,HMGI-C,in benign mesenchymal tumors.' page 885; * abstract * & NAT.GENET. , vol. 10, no. 4, 1995 pages 436-444, -----	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12 March 1996	Delanghe, L

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)